

Science beyond the fringe

It would have amazed the Victorian steadfasts of science how confused some of our attitudes towards science still are. Instead of the logical world they hoped for and tried to work in there is a discernible tendency for the public and even some practitioners of science to turn their backs on science and become preoccupied with the bizarre and the magical.

Mr Uri Geller is only the most recent to cast doubt in the public mind on the efficacy of rational explanation.

Archaeology is being plagued by a series of ideas which have achieved a following particularly among the young. Professor Glyn Daniel has tilted against some of these nonsenses in his fascinating and always readable editorials in *Antiquity*. At one end of the spectrum there are innocuous extrapolations of conventional ideas about the significance of Stonehenge. At the other there are people busily poring over Ordnance Survey maps of Britain plotting mythical alignments between ancient monuments and erecting fanciful hypotheses about prehistoric technological civilisations. It is not as if archaeology needs enlivening—the radiocarbon dating method has already done that and caused great excitement by requiring revisions of old ideas about the relationships between prehistoric peoples.

Elsewhere, Velikovsky is enjoying a revival at a time when real astronomy and the earth sciences have never been more fascinating. How long will it be before psychiatrists are inundated with requests for more research into possession by devils from people who have seen the film *The Exorcist*?

It is difficult to know why these beliefs beyond science have such a following and whether it is genuinely on the increase, but presumably it is closely tied to a prevailing mood of the questioning of established values and a disbelief that science has more than marginal relevance to the human condition.

All this can be fun, of course, but the danger is that taken too seriously it detracts from all the exciting things that are being achieved in, for want of a better description, conventional science based on conventional ideas about the way a scientific investigation should proceed. And, when pseudo-scientific ideas are finally demolished, as they usually are (although one of their characteristics is that they are long in the dying) their demise casts doubt in the minds of the general public about the value of scientific research in general.

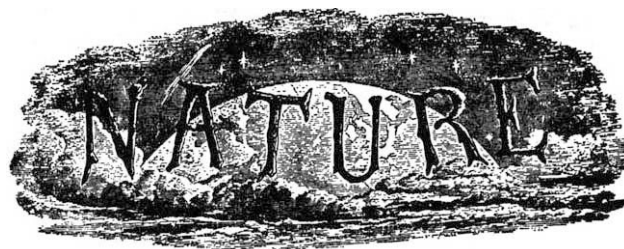
Scientists want to fight this distressing drift from the scientific way of thinking, but are not being noticeably successful. Part of the answer is to make real science more attractive, and for this reason scientists should not be dismayed when they find professional communicators of scientific ideas using imprecise words in place of the

precisely-defined terms from the science laboratory. To capture and convey the excitement of modern science is the main thing. In this respect Professor Kurti's proposal for a code of professional etiquette, published in the November 1973 issue of *Physics Bulletin*, may turn out to stifle scientists' communications with the general public and have the opposite effect to the one he desires.

Nature has a responsibility in all this; a responsibility that perhaps more than anything else guides us in the choice of papers for publication. If the successes of the scientific world are to come through clearly to the general public and appear more compelling than myth and idle speculation does, it is also vitally necessary that the community of professional scientists should understand each other. If scientists, fully aware of the scientific method, find the work of others in different disciplines boring and unconvincing there is little chance that the layman will feel otherwise. As far as we are concerned a scientific journal can help by encouraging its authors to explain their findings in a way that will make them as widely accessible as possible. After all, if a paper is not understandable outside the branch of science in which it originated the paper might just as well be circulated and filed as a private report. It is also a continuous surprise to us how coquettish some authors are when it comes to an assessment of the implications of their findings. Often papers are sent to us describing new measurements of the levels of pollutants and contaminants, but with no estimates of whether the estimated levels are dangerous.

If we are more persistent than most journals in importuning authors to be briefer or blunter this is partly the deeply-felt view that brevity and clarity ultimately lead to wider dissemination of ideas and a greater understanding of science and sympathy with its objectives.

100 years ago



On the Word "Axiom"

IN reference to the controversy between Mr. Spencer and his reviewer about Sir I. Newton's calling his laws of motion "axioms," it is to be observed that there is a certain ambiguity in the word. "Axiom" is from ἀξίωμα (I deem), and would thus signify a first principle to be taken for granted. It does not, of course, carry with it the meaning of a 'necessary judgment which cannot be contradicted.' Whatever may be considered the ground of Euclid's "axioms" so called, Euclid himself did not apply that name to them; but the first nine he called "common notions," and the last three (which are peculiar to geometry) he placed among the postulates (ἐπολογήματα), and heads them with "let it be granted." Now it is clear, from Newton's own words, that in calling his *Leges motus* "axioms," he does not imply that they are *necessary* judgments, but that he requires them first of all to be granted (however established) in order to the following reasoning. In other words, they are postulates, like Euclid's last three "axioms." In our modern use of the words "axiom," "axiomatic," there is always implied the *ground* why a proposition is demanded as granted, viz., because its necessity is self-evident; but this wider use is not required by etymology, or (I think) in interpreting all ancient writings. F.M.S.

From *Nature*, 9, 462, April 16, 1874.



The problem of Soviet scientists: a reply to John Ziman

Professor Eric Burhop, of University College, London, replies in an open letter to points raised in an earlier article on relationships with Soviet scientists.

DEAR JOHN,

Although there may be many things wrong with the organisation of science and the attitude toward some scientists in the Soviet Union, I do not feel they warrant the kind of special action you propose in your article in *Nature* (246, 322; 1973).

In the first place your article is tendentious and contains a number of inferences which I believe you would have difficulty in establishing. It would indeed be a matter of concern if Soviet science "were to fall into decay and disrepute". Of course if, like Solzhenitsyn, we were to hark back a quarter of a century, there might be some cause for concern. But the Soviet scientific community has itself been able to correct the most glaring of the aberrations that gave rise to anxiety at that time. The difficulties it faces today, while no doubt often stupidly frustrating, are not of the kind likely to threaten Soviet science with "decay and disrepute". The reasons Soviet scientists often turn up late at conferences or do not turn up at all is mostly, though admittedly not entirely, due to a clumsy bureaucracy which affects not only scientists. These are the kinds of things—basically trivia, however maddening they may be—that can only be effectively dealt with by the Soviet scientists themselves. I agree that it would be beneficial if a greater number of Soviet scientists were able to come to conferences abroad. I think the position is improving but the problem is very largely one of foreign exchange of which there is a great shortage, affecting all travel. It would certainly be a more sensible use of the available foreign exchange to allow a larger amount for travel of scientists but this is not the kind of thing that can properly form the basis of anything approaching a demarche by the Royal Society.

Some criticisms you make are serious ones with which I would not disagree, but the situation is more complex than you imply. You ignore completely the differences in values characteristic of Western and Soviet society. For example, in Western society the increase of private wealth by speculation is regarded as normal, or even laudable. In Soviet society it is punishable very severely. In Western society too much concern for problems of peaceful coexistence, disarmament and the abolition of nuclear weapons is regarded as at best naive and at worst subversive. In Soviet society these things are highly regarded.

In a society based on social ownership of the means of production, the operation of a planned economy is likely to require more restrictions in some directions than in a society based on the vagaries of the free market. Many people, including I believe many scientists or writers, may feel this a price well worth paying for a society founded on socialist principles, in which continued social, economic and cultural advance is assured. They may indeed assess these as far less onerous than the 'restrictions' inherent in inflation, unemployment and social insecurity which are unknown in their society but all too real in our own society.

Many of the disabilities to which you refer are related to the question of the emigration of scientists to Israel. The Soviet Union has consistently supported the Arab countries against Israel and this policy seems to have very wide support

among Soviet people who tend to regard those Soviet scientists who wish to emigrate at the present time and are presumably willing to help Israel in the war in somewhat the same light as British people on the whole regarded Oswald Mosley in 1939. It may be regrettable, but hardly surprising, that their colleagues have in some cases refused to work with scientists who have applied to emigrate to Israel. This is surely a passing phase and if and when a stable peace comes to the Middle East the campaign against the emigration of scientists to Israel will abate.

Arguing that the Council of the Royal Society should intervene, you say "such a statement would make it quite clear to the main body of Russian scientists that we understand their present position . . .", implying that they would welcome such a statement. I do not believe there is a basis for such a conclusion. I have come into contact with many Soviet scientists and I know that the vast majority would not welcome such intervention. Indeed this has been pointed out to me quite passionately by some eminent Soviet scientists who are by no means complacent about some of the aspects of the situation to which you refer. They feel that an intervention of the type you envisage would certainly make things worse by strengthening more 'hard line' as opposed to more 'liberal' tendencies.

The most important reason for opposing intervention is, however, surely the effect it could have on the development of scientific exchanges. You place very little store on these exchanges. Others, however, have had a different experience of them. The interests of both British and Soviet science require an extension, not a cutting off of such exchanges. There is, however, an even more important reason for fostering international scientific cooperation in general and cooperation between the Royal Society and the Soviet Academy of Sciences in particular. They are an important factor in building relations of friendship and understanding between our two peoples. They can assist in breaking down the fortress mentality which largely colours Soviet attitudes to the outside world. This attitude has been built up over the years as an understandable consequence of political attitudes towards the Soviet Union. It constitutes a major obstacle to the development of the easy, informal relations between Soviet colleagues and their opposite numbers in the West. I know you would like to see these relations develop and yet I fear that the kind of campaign you are sponsoring will have the effect of strengthening rather than loosening this fortress mentality.

For the Royal Society to intervene would represent a departure from long established policy. All through the Vietnam War it said not a word about the way in which modern scientific techniques were applied by the United States Army, assisted unfortunately, by many United States scientists who should have known better, in the development of defoliants, virus diseases of growing rice crops, riot control weapons, the electronic battlefield. In Chile at the present time the military junta has taken over the universities and many university staffs, including scientists, have lost their jobs, have been forced to emigrate, or even put in danger of their lives. Recent press reports make it clear that the United States Government continues its programme of 'behaviour modification' experiments on prisoners—a gross professional abuse of psychiatry, as you rightly say. I have not heard suggestions that the Royal Society should say anything about any of these things.

In my opinion, before the Council of the Royal Society starts making public statements about the matters you raise in your article it might appropriately look at things in Britain. I do not wish to exaggerate but, nevertheless, there are blemishes sufficiently serious for the National Council for Civil Liberties to feel impelled to set up a Council for Academic Freedom and Democracy here.

Yours sincerely,

ERIC BURHOP

international news

A CONTROVERSIAL scheme to explode a string of nuclear devices underneath the Colorado Rockies to extract vast deposits of natural gas has run into a critical snag. A key test of the technique, a 90-kiloton blast called Rio Blanco which was set off last year amid a storm of protest, has turned out to be something of a flop, and officials of the Atomic Energy Commission (AEC) are now trying to find out what went wrong.

The gas is trapped in small pockets in a layer of sandstone about a mile underground, and there is reckoned to be enough under the Rocky Mountains to supply the entire demand in the United States for about 10 years. But the sandstone is not porous enough to allow the gas to be extracted simply by drilling into it, and so the AEC together with an industrial partner, has been trying to free the trapped gas by smashing the rock with nuclear explosives.

The idea is to fire off a nuclear device in the gas-bearing sandstone, which creates a large underground cavern and fractures the surrounding rock for hundreds of feet in all directions. Gas then seeps into the cavern through the fractures and it is later pumped to the surface.

Rio Blanco was the third test of the technique, but it was the first to employ a radically new idea to improve the gas yield. Instead of firing off a single nuclear device, three explosives were placed in a vertical line, about 450 feet apart, and triggered simultaneously. The hope was that the caverns produced by each explosion would link up to form a huge cylindrical chimney in the sandstone layer, about 150 feet in diameter

Rio Blanco draws a blank

Colin Norman, Washington

and 1300 feet deep, surrounded by massive fractures radiating outwards.

But earlier this year, when the AEC began to extract gas from the chimney, it found that it was getting a yield only from the cavern created by the topmost explosion—somehow, the caverns either failed to link up or they had later become sealed off from each other.

Asked last week what went wrong, J. Keith Davey, an AEC official concerned with the project, said that several things could have happened. The simplest explanation is that the explosives were placed too far apart so that the caverns they created just didn't join up, but another possibility is that the chimney could have become blocked.

It is possible that the glassy melt formed by the intense heat of the nuclear explosions didn't fall to the bottom of the chimney, but blocked off the connection between the caverns. Another likelihood is that the rocks simply shifted after the explosions as Davey points out, underground mechanics at that depth are not well understood. Still another possibility is that the chimney could have been disturbed when the borehole was sunk into it to pump the gas to the surface, a suggestion which is given some credence by

the fact that about 12,000 barrels of lubricating mud were lost during the reentry operation.

Later this year an attempt may be made to sink an oblique shaft into the second cavern to try to provide some answers, but whatever went wrong the failure has greatly damaged the chances that nuclear gas stimulation—as the technique is called—will ever be turned into a commercial venture. The scheme has already run headlong in spirited opposition from several diverse groups, and technical problems at this stage could well send it to an early grave.

Rio Blanco itself was entirely an experimental test, but what is worrying opponents of nuclear stimulation is the prospect of a full-fledged commercial venture. To exploit the gas field under the Colorado Rockies entirely would require several thousand nuclear blasts, a prospect which pleases neither the people living in the area nor the oil companies who are hoping to produce oil from the shale fields which happen to sit immediately above the gas-bearing sandstone.

A repeat of the Rio Blanco test would be sure to attract a huge amount of opposition, particularly in view of the fact that two oil companies have just paid nearly \$210 million for the right to set up a prototype oil shale process on a small strip of federal land not far from the Rio Blanco test site. Alternatively, the AEC would be hard put to justify moving to a pilot scale nuclear stimulation operation on the basis of the Rio Blanco results. The failure is thus a critical blow to the whole enterprise.

Cash on the rail

Rail from Ascension Island, drawn by the explorer, Peter Mundy, 1656



WHEN does specimen collection of rare birds and animals for scientific study become unacceptable? This question has been exercising ornithologists on both sides of the Atlantic recently. The trouble seems to arise when enthusiastic ornithologists and museum staff visit remote places and offer cash and other inducements to the natives for rare birds dead or alive.

The most recently reported case concerned a member of the American National Museum of Natural History of the Smithsonian Institution, who, during a visit to St Helena for the admirable purpose of studying the fossil remains of flightless rails, admitted offering cash rewards after the natives re-

ported the presence of a living bird of this type. No specimen was in fact forthcoming and the author says that he is convinced that the bird was imaginary. But he might remember Charles Kingsley: "no one has the right to say that no water babies exist, till they have seen no water babies existing; which is quite a different thing, mind, from not seeing water babies".

Elsewhere in his article, the author says that the flightless birds of these remote islands were certainly only made extinct after men arrived. The Smithsonian seems to be following the Victorian idea that an animal is not discovered until it is safely stuffed in a museum.

Around the Solar system in 1,800 days

FOLLOWING the complete success of Pioneer 10, NASA has decided to re-target Pioneer 11, now en route for Jupiter, rather than simply to duplicate the earlier mission. As a result, Pioneer 11 will now swing by Jupiter, using that planet's gravity to assist it into a new orbit and a rendezvous with Saturn late in 1979. And that will pave the way for the planned Mariner missions to Jupiter and Saturn, which include the possibility of putting at least one of the Mariner craft in orbit about Saturn.

Pioneer 11 has now passed the asteroid belt and is more than 4.5 astronomical units (AU) from the Earth; its new path should take it to an encounter with Jupiter on December 2 or 3 this year. The latest Pioneer will pass much closer to the cloud tops of Jupiter than its predecessor (27,000 miles as against 81,000 miles, according to a report in *Aviation Week & Space Technology*, 100, 18; March 25, 1974). This close approach is essential if good use is to be made of the slingshot effect of Jupiter's gravity and using such approach can only be contemplated in the light of the Pioneer 10 data. Those data showed the structure of magnetosphere and radiation belts sufficiently clearly that the Pioneer project scientists are confident that Pioneer 11's new path will take it through a gap in those belts, so that in spite of its close approach to Jupiter it will receive less damaging radiation (by some 75%) than did Pioneer 10.

But the actual path followed by Pioneer 11 after the Jupiter encounter is almost as interesting as the objective itself. Like the recent Mariner 10 Venus/Mercury mission, which was the first to utilise a gravity-assisted trajectory, Pioneer 11 will actually be slowed down by its encounter with the first planet it passes (Jupiter). The new path will take the Pioneer across the leading edge of the planet (Pioneer 10 passed the trailing edge and was accelerated above escape velocity for the Solar System) and in a loop back

across the Solar System, to meet Saturn, eventually, on the opposite side of the Sun from the Jupiter encounter.

The slow journey back across the Solar System will take nearly five years, with Saturn being reached some time in October 1979. The closest approach to the Sun will be some 3.5 AU but the space probe will not have to face the hazard of the asteroid belt again, since its new path will take it out of the ecliptic plane. Clearly, this will provide a wealth of information about interplanetary space. But what are the prospects for the Saturn encounter itself?

Saturn orbits some 9.5 AU from the Sun, at which distance radio signals take 1.5 h to reach Earth. It would be asking too much to expect spectacular information to be relayed by the first probe to visit Saturn—but as long as the radio transmitter is still functioning useful data could be obtained simply by flying Pioneer 11 through the rings of Saturn to see if it hits anything. That would at least indicate the limits of safe orbits for the following Mariner craft.

It looks as if that at least should be possible, and some scientific information may also be gleaned as a bonus. The spacecraft's nuclear isotope generators were designed to produce 100 W five years after launch (April 1978 in the case of Pioneer 11) but judging from the experience gained by Pioneer 10 the generators are likely to last rather longer than anticipated. It seems feasible that all the experiments could still be operating in October 1979, with sufficient power available to relay their findings, and perhaps even pictures, over Pioneer 11's 10-W transmitter.

FOOTNOTE: It seems that the *Sunday Mirror* has even more faith in the versatility of NASA spacecraft and the power of the gravity-assist technique than NASA project scientists themselves. In the March 31 edition of that newspaper we were presented with a Mariner 10 picture of Mercury with a caption which ended "Mariner 10 is now hurtling through space ready to take pictures of Saturn, the ringed planet . . . in 1979".

Mars Sampler in 1981?

NASA engineers and scientists will be meeting in Washington on May 23 and 24 to discuss the feasibility of a unmanned Mars sampling mission. Such a mission could be flown as early as 1981 if there is cooperation between Soviet and American space agencies.

The prospect of complete collaboration between the two giants has been growing for some time, and the Mars project seems a logical next step. The mission divides clearly into two parts; one country could take responsibility for the launch from Earth and delivery to Mars of the sampler package while the other looked after that package and produced the means of getting the sample home.

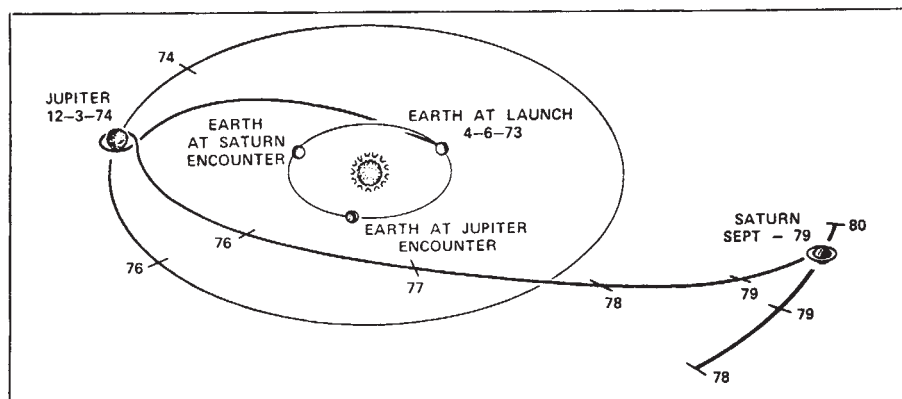
Some of the possibilities which will be trashed out in detail in May are mentioned in *Aviation Week & Space Technology* (100, 14, April 1, 1974); support for the proposal is likely to be strong both because of the desire to develop from the 1975 Viking lander (assuming that is successful) and because the returned package need only be placed in Earth orbit, where it could be collected by the shuttle. But options are being kept open on such projects as Viking 1979 (*Nature*, 248, 15; 1974), and it remains to be seen whether the Russians would accept the proposal of a joint mission to bring back Mars samples.

Ring of little confidence

John Gribbin

SHOULD the Science Research Council (SRC) support the building of an electron storage ring at Daresbury to provide a central synchrotron facility when the electron accelerator NINA closes down in 1978? Taking advantage of a spare afternoon at the end of a conference on synchrotron radiation held at the University of Reading under the auspices of the Institute of Physics, the SRC case study and plans for such a facility were put to the physics community on April 2. Because of a clash of dates with a Chemical Society meeting, the proposal has still to be discussed with rank and file chemists. But the Reading meeting showed clearly that at the very least the proposal needs careful rethinking and emphasised the value of such discussions with the people who would be using such machines.

As at present conceived, the new machine would cost some £2 million to



build, with running costs in the vicinity of £750,000 a year. For this money, the facility would provide the first such source in Britain dedicated to synchrotron work—present synchrotron users at Daresbury and elsewhere are very much aware of their status as 'parasites' on the high energy machines.

Ten beam lines, each capable of supporting two projects, would eventually be available but it would take some 10 years to work up to this fully operational capacity. The machine would operate at 2 GeV with an electron current of 1 A, the planned radius of the electron orbit being 15 m, the bending radius of the magnets 5.55 m, the magnetic flux 1.2 T (12,000 gauss) and the total radiated power 240 kW. All that is impressive enough, as is the list of possible experiments which could be carried out with such a facility. But is it the best way to spend the available money?

Many of the physicists present at Reading expressed doubts about this. The most crucial question, which is not yet answerable, is just when the facility could begin operations. Both for economic reasons and because of the community which has built up around Daresbury, the plan envisages that the new ring would be built inside the NINA hall. Inevitably, that means that there must be a delay between NINA closing down and the new machine becoming operational. The optimists suggested, in the SRC plan, that the delay might be a year or so, provided work begins on the project soon. But few, if any, of the synchrotron users believed this. B. Bleaney (University of Oxford) went so far as to say that he would bet on a gap of more like four or five years in which British synchrotron workers would have no major facility in this country; there were no takers prepared to accept his bet.

That raises the question of just how useful such a machine will be in the mid-1980s. Certainly everyone present at Reading would have been delighted to have such a facility now but fears were expressed that European and American machines now being planned and built might have tackled the most exciting problems before the new British machine got a look in. Of course, such machines will be extremely useful for the foreseeable future, just as conventional X-ray sources, for example, have not been rendered obsolete by synchrotron facilities. But it is one thing to build such a costly installation, do the exciting work and then continue using it into a productive middle age, and quite a different kettle of fish to build an expensive facility knowing that it will probably only ever be used for routine, if valuable, work.

There was also some confusion about just how much the costs would be, in terms of experiments using the facility.

It was put to the SRC representatives that although their plan did not spell out the financing in those terms, a generous interpretation of the running costs, together with the proposal that ten "new projects" might start up each year, meant that each project had to be "worth" £100,000. That is a lot of project—something like three or four times the cost of typical projects being run in universities in the areas of research expected to benefit from the new facility. Even allowing for Parkinson's laws, is it really sensible to plan for such growth?

But there are two points to be considered against this apparently large expenditure. First, if only 100 people are using the machine, then its running cost of £750,000 a year breaks down as £7,500 a person a year—more the sort of figure which the average person can understand and, it seems, not a sum to make the SRC blanch. Second, at present, experiments funded by the SRC in universities have invisible means of support—they do not have to pay for buildings, for electricity, for non-scientific staff to keep the buildings running and so on ("heat, light and sound", as a wag in the audience put it). And in the present economic climate it is best, perhaps for the SRC to be seen to stand on its own two feet.

This problem, although slightly out of the mainstream of the Reading meet-

ing, is an interesting and tricky one. For, if the SRC grasps the nettle firmly and says publicly that its expenditure plans are based on the assumption of less university support, such bodies as the University Grants Committee will surely take note and reduce their allocations accordingly. On the other hand, if the SRC assumes continued support of this kind at present levels then it will be severely embarrassed if such support is not forthcoming.

It is certainly good to see the SRC encouraging open discussion at the early stages of planning such projects as the new storage ring and it was clear that both the SRC and the physicists were able to learn about each others' difficulties and needs at Reading. The plan as originally envisaged seems unlikely to survive and a powerful case can be made for pressing ahead more vigorously with such a project, perhaps at a different site, if there is to be such a ring at all. It also remains to be seen how the need for such a large sum might inhibit other activities by the SRC's Physics Committee—it is not not difficult to think of ways of spending £2 million which would be at least as useful as building a synchrotron facility. But certainly there is less chance of ending up with an underused white elephant when the committee is taking such a receptive attitude to frank comments from the physics community.

Business report *Roger Woodham*

A HEALTHY increase in capital has been announced by Imperial Chemical Industries (ICI), following the publication of its annual report for 1973. The amount sanctioned is £250 million, 60% of it earmarked for the United Kingdom where the major project started in 1974 may well be a giant ethylene plant on Teesside producing 500,000 tons a year. This was originally to have been a three-way affair—Shell, ICI and BP—but Shell has now withdrawn for undisclosed reasons and a firm agreement has not yet been made between ICI and BP. Will intercompany squabbles spoil the attempts to avoid a recurrence of the plant overcapacity problems of recent years?

If all goes according to plan ICI will be livening up its investment in spite of Mr Healey's Budget. Just how things have ground almost to a halt recently is highlighted by figures in the 1973 report for fixed assets—plant, equipment, buildings and so on, the stuff of investment. In 1973 fixed assets increased from £1,160 million to just £1,185 million. By contrast, in 1964, 1965 and 1966 the assets in use were increasing by leaps and bounds, from £646 million to £760 million to £880 million.

The report also reveals some interest-

ing trends in ICI's sales, worth £2,166 million last year. Whereas in 1963 the United Kingdom accounted for 52% of the sales, in 1973 the proportion was down to 43%. Allowing for imports of raw materials and so on, ICI contributed positively to the British balance of payments by some £220 million.

●HELICOPTERS landing on oil rigs, and possibly ships entering estuary ports, may benefit in a few years from the improvements that continue to be made at the Mullard Research Laboratories, Redhill to its Microwave Aircraft Digital Guidance Equipment (MADGE). The basic system has NATO's blessing and is being manufactured commercially.

A substantial proportion of one of MRL's four divisions, which between them spend £3 million a year, is engaged on work of broadly this kind, sophisticated radar. The beauty of MADGE is that the stationary part of the system, which is 'interrogated' by a microwave signal from an aircraft (or ship) can be set up in only 15 minutes or so. Ideal, as NATO evidently thinks, for landing and takeoff near the front line, but also a good bet for civil aviation.

LATE LAST year, when Arab countries suddenly shut off oil supplies to the United States, the Nixon Administration and the United States Congress reacted predictably by espousing a pell-mell drive to make the United States independent of foreign suppliers of energy so that the country would not get caught short again. President Nixon dubbed the effort "Project Independence", and optimistically said that the goal of self-sufficiency could be reached by 1980—a prediction which he continues to make even though his own officials have publicly stated that it is totally unrealistic.

A striking aspect of the debate about Project Independence is that it has centred almost entirely on the levels of funding that have been proposed and on the way the project should be managed, but few people have publicly challenged the philosophy behind the enterprise. Like God, motherhood and apple pie, energy self-sufficiency is tacitly assumed to be good for America. But a report published last week by the Ford Foundation may change all that.

A preliminary publication designed to stimulate debate about the energy policy choices that now confront the United States government, the report says that the decision "to rapidly develop all federal energy resources simultaneously . . . may make sense in some areas, but as an across-the-board proposition it represents a failure to weigh conflicting values". For one thing, it may result in unacceptable environmental degradation, and for another, it may have damaging repercussions on United States foreign policy. Moreover, the report points out that Middle East oil can be produced at very low cost, and although at present prices it may seem economic to extract oil from shale and coal, there is no guarantee that these products would not be undersold by Arab oil in an open market.

Prepared by the Ford Foundation's Energy Policy Project—a \$4 million study which should be completed later this year—the report is an attempt to suggest that the United States is not "bound in an energy straitjacket" but has a number of policy choices available, ranging from a gradual progression to zero energy growth on the one hand, to aggressive development of new energy resources, so that Americans can continue to increase their consumption of energy virtually unchecked, on the other, the report outlines some of the social consequences of the options.

If the United States continues to increase its energy consumption at the same rate as in the golden years preceding the crisis—about 3.4% a year—production will have to be doubled by the year 2000. According to results of a study carried out by Resources for the Future, Inc., such a target could be met by an aggressive programme of

developing domestic sources of energy, including oil from the outer continental shelf, nuclear technology and shale oil. There is, however, some question about the availability of fossil fuels much beyond the year 2000 if they are exploited at maximum capacity for the next 30 years, and the energy demand could only be met by abandoning some environmental standards.

At the other extreme, the United States could move gradually towards

Ford spells out the energy choices

Colin Norman, Washington

zero energy growth by developing more efficient ways of using energy and by cutting back on consumption. If such a path is followed, it would mean some rather fundamental changes in the American way of life, but the report suggests that the goal could be met without dire consequences, either to individuals directly or to the economy of the United States. In fact, the report states that "because of its greater efficiency in using energy, the [zero energy growth] scenario would include many more energy benefits than the nation now enjoys".

A key to this optimistic prediction is an orderly progression to zero growth, rather than a sudden dislocation, so that the goal will be reached towards the late 1980s. By that time, individual energy consumption would have risen by about 10% from the present level. "Managing a transition to zero energy growth appears to be possible if the change takes place gradually over ten to twenty years as part of long term planning and a growing social consensus as to its desirability", the report states.

To achieve the goal of zero growth changes must be made—for example people would have to spend less time travelling, efficient rapid rail links would have to be developed between cities, there would have to be a trend toward multi-family housing, the growth of energy-intensive industries would have to be slowed down, there would have to be more recycling of valuable products such as aluminium and the use of plastics would have to be curtailed. Clearly, such changes will not come easily and will demand a fundamental change in governmental thinking to ensure that an orderly transition is made to "a society that husband its resources, including energy".

Between those two extremes lies the possibility for increasing energy production while reducing the growth in demand. This scenario is called the "technological fix scenario" in the report and in a free enterprise economy it is probably the most likely course for the country to follow. To illustrate the choices available, if such a path is followed, the report outlines some of the decisions which will have to be made to cut the rate of growth of energy consumption to half that pertaining before the energy crisis began to bite.

One fundamental fact to emerge is that although some research and development will be required to develop technologies for conserving energy—such as solar heating and cooling, and heat pumps for residential heating—"the major uncertainties are political, institutional and economic questions rather than physical or technical limitations". Which is a little like saying that the United States could move from a capitalist to a socialist economy but for political constraints.

Another important aspect of the technological fix scenario is that with an aggressive programme of energy conservation, choices can be made between competing technologies for energy supply. For example, the report points out that if strip mining of coal in the western United States is considered to be too destructive to the environment, other sources of energy could be expanded to fill the gap.

Alternatively, the report mentions in several places that fundamental questions have arisen in the United States about the safety and environmental risks associated with nuclear power, and it suggests that a moratorium could be called on the building of new power plants until such issues are resolved. Since the United States Atomic Energy Commission (AEC) is basing its case for the fast breeder reactor chiefly on the fact that it will supply a huge proportion of the country's energy requirements by the end of the century, the AEC will not take very kindly to the suggestion that the entire nuclear programme could be abandoned without plunging the United States into total darkness.

The fact that such options exist has largely been closed over in the debate about Project Independence, and the chief result of the report will be to strengthen the hand of environmental groups in their opposition to some of the more damaging energy projects. But this document is simply a discussion device, and the facts behind the analysis will not be published until late this year. In particular, the final report will present a more closely argued analysis of the consequences of a transition to zero energy growth.



Professor R. V. Jones. A wartime picture by Larry Burrows

Concurrence in learning and arms

David Davies

It was the reunion of the Class of '45 at a recent meeting of the Royal Society to discuss the effects of two world wars on the organisation and development of British science. No gathering of reminiscing fighter pilots could match this occasion, where the boffins, with their H2s, operations research, radar and the Manhattan project, assembled once more to draw the morals, not in a smoky bar room but in the clinical jumbo jet interior of Carlton House Terrace. Two Lords, two Members of the Order of Merit, seven knights and all Fellows of the Royal Society, except the educator, the historian and the industrialist. The high-spot seems to have been the night before when the speakers gathered round the dinner table to swap stories—not, one of the participants pointedly told me, the sort of stories to be repeated within earshot of the Editor of *Nature*. Nevertheless there was sufficient vigour left on the day to delight an audience of two hundred.

Before the twentieth century war had practically no impact on science, nor science on war. R. V. Jones (who had convened the meeting) named the few exceptions—Bacon had advocated "concurrence in learning and arms", Babbage and Playfair, the extraordinary precursors of so much, had concerned themselves with science in warfare and Kelvin and Rayleigh advised the services. Organised science was still small; the Cavendish was getting by on £1,500 a year just before the First World

War (WWI), including demonstrators' salaries. There were terrible shocks to British complacency in WWI—gas, Zeppelins and U-boats were three scientific and technological tools of war which had to be countered. Science was also badly hit by the loss of Moseley and Hopkinson. Scientists were wasting their talents as cannon fodder and in the Second World War (WWII) a register of scientists was maintained.

The successes of WWII, particularly radar, finally ensured that science was moved closer to policy making. Lindemann was the first scientist since Playfair to be involved at governmental level and the need to share scientific results led to the dispatch of A. V. Hill, Ashby and Needham to Washington, Moscow and Chungking.

It is easy to see the importance of WWII because many of the participants are still around but WWI had an equally profound effect on science, as D. S. L. Cardwell pointed out. Germany had encouraged science-based industry and Britain's dependence on Germany in such matters as dyes, pharmaceuticals, instruments, fine chemicals and so on led to grave shortages. New ventures were launched—British Dyestuffs, research associations for industry and the Department of Scientific and Industrial Research (DSIR)—and by the end of the war the importance of science in education was realised. British science got a leg up from WWI but science in general lost much of its supranational character.

What sort of leg up did science get from WWII? This question occupied speakers for the rest of the day. The Second World War, it seems, gave a boost to scientists rather than science. For science itself there were obvious

gains such as nuclear physics, but also less obvious losses (C. H. Waddington held that without WWII molecular biology would be fifteen years older, Bernal and Astbury, not Watson and Crick being the midwives). In technology too there were both gains and losses, as D. S. Davies (ICI) detailed in a thoughtful paper. Prewar inventions such as titanium technology, jet engines and radar were clearly advanced but against this were setbacks in television, synthetic fibres and plastics. A static gross domestic product inhibited investment in special plant in the war, of course. Of specifically wartime inventions, maybe operational research was the only one which has so far had a major impact on Britain.

The effect on the scientists had, however, been colossal, both in the way they went about their science and in the way they ensured that government took note of them. J. A. Ratcliffe and Sir Edward Bullard told broadly similar stories. The scientist who in 1939 left a laboratory used to making do with no phone and no secretary, used to the idea that science was gentlemanly, cheap and simple and used to being rebuked by the chief assistant for using expensive wood to make breadboards ("mahogany, Mr Ratcliffe?") came back a changed man. He had learnt how to get his way, how to coordinate a team and how to use communications. The outward sign of this was the growth in numbers of research workers and the even more spectacular growth of the cost of research projects. As for biology, even though, as J. W. S. Pringle put it, this was not a biologist's war it changed the thinking of many who found pure research not so important and the possibility of doing new things and playing a role in public affairs attractive.

It was inevitable that American science should have come in for mention. Before WWII, said Lord Bowden, America had been regarded as subordinate to Europe in science but the vision of men such as Vannevar Bush and a willingness to buy scientists, if necessary, swung the pendulum. Besides, nuclear energy, the first big science, was growing and, as Sir Harrie Massey described, the Manhattan project sowed the seeds for a whole new approach to scientific operations in which the United States had a clear start. The Second World War had given many scientists a sense that what was possible in principle was also possible in practice. In wartime, of course, this did not have to be profit making; the carrying over of this attitude into peacetime has not necessarily led to the science-based industries hoped for.

R. V. Jones considered government establishments in a second paper. The aim before WWII had been to keep

government and university scientists close together, but after WWII, despite early hopes that teaching and fundamental research could have priority over defence research, the government gained at the expense of the universities.

In 1931 there were 1,053 civil service scientists. By 1961 there were 15,474. Jones was critical of the trend and contrasted it with the United States, where nongovernmental laboratories had been fostered in the 1940s. Overenthusiastic claims for the Comet plane, thermonuclear fusion, the Einstein redshift in the Mössbauer effect and carbon fibres had not helped the reputation of the

establishments. In wartime they flourished because of the spirit of collaboration. Not so in peacetime when the spirit was competitive.

Finally, how did scientific advice to the government change as a result of the war? Lord (*alias* Solly) Zuckerman sketched the transition from the advice, often informal, of the thirties, of Lindemann, Tizard, Blackett, Bernal, and so on, through the war when many scientists became planners with operational research and a few were involved in the determination of national policy, to the post war period with the greatly enhanced reputation of the scientist in government. Science advisers were

brought in all over the place but their utility was restricted as they were rarely allowed to be more than technical assistants, kept out of the bigger issues of government. This trend culminated in the creation of a Chief Scientific Adviser in 1964, though if there were an issue on which he and trade union leaders had something to say, the trade unionists could be expected to carry more weight.

A fascinating meeting. Too bad the youngsters chose to stay in their laboratories. Those with scant regard for the history of science as a proper study (and there are many) would have been won over by this delightful occasion.

correspondence

Nuclear simplicity

SIR—The paramount command for nuclear power reactors is safety. The next most important requisite is the lowest possible cost of the electric power actually supplied to the grid.

Safety depends directly on simplicity and so does the cost of safety. An inherently simple plant can be made safe more easily and at a lesser cost. Simplicity also favours high availability and thus a lower unit cost of electrical power.

Simplicity is rare because new ideas are rare. The original safety containment concept was based on countering the hazard of brute force by brute strength until, quite late on, somebody hit on the idea of providing a condensational pool. The same is true of the coolant recirculation pumps: first external pumps with large connecting pipes, then external pumps with internal jets and small pipes, then axial internal pumps without any pipes. Pipes may fracture. In both cases the simpler and, with hindsight, very obvious concept resulted in lower costs and much greater safety.

I owe the concept of ponnery to my children who used to build edifices of chairs, saucepans, the cat's basket, vacuum cleaner and so on, up to the ceiling, one 'pon the other. Such an edifice is called a ponnery. I now classify nuclear reactors into 'naturals' and ponneries.

The naturals are simple and obvious. I know of only two: the boiling water reactor (BWR) and the gas-cooled fast breeder (GFB). The pressurised water reactor (PWR), the heavy water reactor (HWR) and the sodium-cooled fast breeder (SFB) are ponneries.

The BWR works on a direct cycle.

Disadvantage: active live steam. The main activity (^{16}N , generated by activation of oxygen) is, however, so short-lived that only the head end of the turbine need be shielded. Advantages: inherent, very rapid reactivity control (voids ratio), so that load follow-up is automatic and power excursions are self-limiting. No primary/secondary heat exchangers, so, no unnecessary thermodynamic losses and no leaky tubes, therefore high availability.

The PWR is, by comparison, a ponnery. It has a complex primary cycle, huge primary/secondary heat exchangers (very expensive and very leak-prone) and a low thermodynamic efficiency factor. High primary pressure (expensive), very modest live steam pressure (inefficient). From the nuclear point of view, sluggish reactivity control, hence the need for boron poisoning, hence a further sprouting of the ponnery to take the boron out again. It is inherent in a ponnery that the more you try to improve it the more profusely it sprouts.

The HWR introduces yet a third compartment, leaks from which must be controlled particularly carefully because heavy water is expensive. This is a ponnery within a ponnery. Its only advantage is the use of natural uranium. Once European enrichment facilities become available, this issue will be dead.

The obvious successor to the BWR is the fast breeder. But which one? The SFB is an obvious loser. It is of necessity a three-cycle system. Sodium is not a natural answer for transferring heat to water.

The natural winner seems to be the GFB, especially at high temperatures (process heat) and in combination with helium turbines (direct cycle, high effi-

ciency, minimum waste heat dumping problems). The technological problems associated with high temperatures (950°C and more) are worth solving, in contrast to those of the SFB. In its prime, perhaps 10 years from now, the direct cycle GFB should prove even simpler than the BWR.

Yours faithfully,

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Fishy correlations

SIR—In reference to the article *What's in a Name?* (*Nature*, **246**, 385; 1973) and G. Curzon's letter (*Nature*, **247**, 82; 1974) citing contributions to this field by brain research workers, the pioneering work of American ichthyologists must not go unmentioned¹⁻⁴. Indeed the more than 300 ichthyological publications of Theodore N. Gill (1837-1914) make him perhaps the most productive investigator in this esoteric science.

Yours faithfully,

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¹Fish, F. F., *Furunculosis in wild trout*; *Copeia*, 1937(1), 37 (1937).

²Fisher, G. C., *Salt-water minnow in fresh water*; *Copeia*, 1920(79), 18 (1920).

³*A complete treatise on artificial fish-breeding* . . . (edit. by Fry, W. H.), (New York, 1854).

⁴Pike, N., *Notes on fishes of the ocean*; *Trans. R. Soc. Mauritius*, n. s. 7, 27 (1873).

news and views

When did plate tectonic processes begin?

A POINT often underemphasised by earth scientists, presumably because they think it too obvious to mention, is that their view of the Earth's behaviour is still heavily biased by data relating to the comparatively recent past. That the bias exists is, always has been, and probably always will be, inevitable, if only because younger rocks are generally the more abundant, the less altered from their original states and the more easily associated with processes whose occurrence is beyond doubt. But there are dangers in it nevertheless. For one thing, the more biased the results towards a relatively restricted range of time, the greater is the inclination to succumb to an uncritical belief in some uniformitarian world view and all that implies in terms of extrapolating the present into the past. It is perhaps significant that as geologists have become more familiar with the pre-Mesozoic and even the pre-Palaeozoic they have become less concerned to defend uniformitarianism or actualism (between which nine out of ten earth scientists cannot tell the difference anyway) and more confused about what these terms really mean or ever meant (except in terms of a reaction against biblical catastrophism). As a result, 'uniformitarianism' has now come to represent little more than a vague attitude, a negative thought that whatever uniformitarianism is it is not catastrophism. On the other hand, so strong is the historical influence that what may seem to be no more than an outmoded philosophical idea may still have deep-rooted behavioural repercussions.

The attempt to extend plate tectonic concepts further and further into the past is a good case in point. The widely-accepted group of ideas known collectively as the new global tectonics is based on data which relate to only the past few hundred million years, or less than 5% of the Earth's history; yet these concepts have succeeded in converting most earth scientists from a belief in a static Earth to a conviction that the planet is on a state of more or less continuous mobility. But however impressive and conclusive the evidence, such a radical transformation is the world view based on such a small proportion of the Earth's existence inevitably raises the question of what is the Earth's 'normal' long-term behaviour. Is the present and very recent mobility of the Earth typical of much or most of the planet's existence, or is it a very late development? Or to put the question in the way it is more usually posed: are plate tectonic concepts applicable to pre-Mesozoic time or not? It is a question every bit as, if not more, fundamental than that raised by Wegener at the beginning of this century.

It is in the nature of things that it should also be a difficult question to answer. What can be said immediately is that if plate tectonics is taken to imply the relative motions of land masses, the extension of the concept to the pre-Mesozoic is inherently implausible. The breakup of the ancient supercontinent of Pangaea some 200 million years or so ago is now well established, and the subsequent motions of the resulting continents have been charted by palaeomagnetic and other means. Finding the physical mechanism for the breakup and subsequent drifting is, of

course, a more difficult matter; but causes apart, the basic idea of continental dispersal from a single land mass poses no severe conceptual problems. By contrast, the proposition that Pangaea itself formed by the amalgamation of many land masses (presumably different in shape and number from the present continents) is conceptually altogether more difficult to accept, and the mechanism for such a union is unimaginable. It would be as easy to accept as, for example, the suggestion that meteorites may accrete in an environment comparable to that in which they disintegrate. That two, or even several, continents may collide and coalesce is not beyond the bounds of comprehension, but that all pre-existing land masses should combine into one can hardly be envisaged. Yet this is precisely what is envisaged by many who have sought to substantiate pre-Mesozoic plate tectonics.

It is at this stage that the dangers of a too uncritical acceptance of some basically uniformitarian viewpoint readily become apparent. It is worth pointing out, for example, that the close association between continental drift (that is, the relative movement of land masses) and plate tectonics at the present time does not necessarily imply that pre-Mesozoic plate tectonic processes would involve drift. In a system of plates in which only the plate containing Pangaea was continental, Pangaea could move relative to the poles as a single land mass; and proof of no relative motion between Pangaea's component masses would not necessarily disprove the existence of plates. This would avoid the problem of the inherent unlikelihood of all land masses coalescing; and the fundamental mobility of the Earth during the pre-Mesozoic need not be rejected.

On the other hand, it must be admitted that the supporters of pre-Mesozoic plate tectonics have often come to that view not directly through a simplistic uniformitarianism requiring the permanence of continental drift, but through an altogether different, though perhaps equally simplistic, uniformitarianism involving pre-Mesozoic drift as a precondition. Specifically, they have argued that modern orogenic belts are the direct result of plate interactions, that the characteristics of ancient orogenic belts are similar to those of more recent ones, and therefore that ancient orogenic belts are the result of ancient plate interactions—in particular the suturing of previously distinct crustal plates which have converged. Africa, for example, contains a pattern of stable cratonic blocks with intervening orogenic belts ranging in age from more than 3,000 million years to Palaeozoic. The argument here then is that the orogenic belts were formed at plate margins, the plates being the cratons which drifted together from positions thousands of kilometres apart.

But although the evidence for this view seems quite strong and is well supported by data from other parts of the world, most notably North America, there is also convincing support for the older, alternative model in which the cratons have remained essentially stationary relative to each other and the orogenic deformation developed between them. There is also a great deal of evidence which at various times has been taken to favour both the stationary and drifting craton models. The fact is that the available data are conflicting; and it seems unlikely that the question will ever be resolved on geological grounds alone. On the other hand, such are the pressures to interpret the past in terms of the present, and such is the strength of the new global tectonic hypotheses, that the case for pre-Mesozoic plate

tectonics has often contrived to appear overwhelming. As recently as last year, for example, McElhinny (*Palaeomagnetism and Plate Tectonics*, Cambridge University Press, 1973) could conclude that it "now seems likely that the processes of plate tectonics have been a feature of all geological time" while at the same time admitting the paucity of palaeomagnetic data from the Precambrian.

That this conclusion may be premature was recently brought home sharply by Piper *et al.* (*Nature*, **245**, 244; 1973) who argued that, whereas the geological evidence may be inconclusive, "palaeomagnetic evidence is potentially decisive". They then concluded from the available palaeomagnetic data that the principal cratonic areas of Africa were probably in roughly their present relative positions as early as 2,200 million years ago and thus that the adjacent orogenies were probably ensialic. More generally, they were able to suggest that the concentration of all existing land masses in one large continent during the late Precambrian remains a serious possibility.

Later in this issue of *Nature*, McElhinny *et al.* present new palaeomagnetic data from Australia and India; and although their interpretation of these and the older data differs from that given by Piper *et al.*, they nevertheless reach the same general conclusions. It thus begins to look more and more as though the inherently implausible never happened. On the other hand, it must be stressed that even now pre-Mesozoic, and especially Precambrian, palaeomagnetic data are still far too sparse to enable any conclusion from them to have the degree of certainty attributed to comparable conclusions relating to the past 200 million years. But what is now certain is that only palaeomagnetism will be able to resolve the issue one way or the other.

P.J.S.

Origin of asteroids

BETWEEN the orbits of the planets Mars and Jupiter resides a collection of minor bodies known as asteroids. They were first discovered in 1801 by the Italian monk Giuseppe Piazzi and caused considerable excitement because their mean solar distance of 2.8 astronomical units fitted neatly into the Titius-Bode series of planetary distances. More than 3,000 have now been discovered and it is estimated that ten times that number may well be susceptible to photographic detection. They are all small, Ceres the largest being about 770 km across, and having a mass of about one five thousandth that of the Earth. In fact the total mass of the asteroids is estimated to be only twice that of Ceres. They also seem to be highly irregular structures, their internal gravitational force being much too weak to make them spherical.

Where do these asteroids come from? Three theories are presently in fashion, the oldest, first propounded by Olbers more than 150 years ago, has the asteroids originating from the cataclysmic disruption of a planet which had an orbit between those of Mars and Jupiter. This received a new lease of life when Ovenden (*Nature*, **239**, 508; 1972), on the basis of his principle of least interaction action, found that he could explain the present planetary positions if another planet ninety times heavier than Earth disappeared between Mars and Jupiter some 16 million years ago.

A second theory (Kuiper, *Astron. J.*, **55**, 164; 1950) produces asteroids by the collisional fragmentation of a small number of planetesimals, these planetesimals being the primaeval condensates from the solar nebula, the fundamental building blocks of the Solar System. The present Hirayama families of asteroids with similar orbital elements are supposedly produced by the fragmentation of individual planetesimals.

Theory three (Alfvén, *Icarus*, **3**, 52 and 57; 1964) has the asteroids accreting by random aggregation. As the bodies are inelastic, collisions tend to reduce relative velocities and thus to equalise orbits. The particles always return to the point in space of their last collision leading to collisional focusing and the production of a stream of particles of very similar orbits and low relative velocities—an ideal situation for accretion. If this theory is correct the asteroids are in the process of growing into a planet with a mass similar to that of Mercury.

Napier and Dodd (Royal Observatory, Edinburgh) in a recent edition of the *Monthly Notices of the Royal Astronomical Society* (**166**, 469; 1974) have tried to discriminate between these theories by considering the observed mass distribution of the asteroids, the observed rotation periods and also the physical tenability of the processes required.

The number-mass distribution of asteroids smaller than 20 km obeys a power law where dN , the number with masses between m and $m + dm$, is given by $dN \propto m^{-s} dm$. Dohnanyi analysing the data obtained from the Palomar-Leiden survey of asteroids finds them to have an s value of 1.84; Hellyer found $s = 1.77 \pm 0.05$ using the same data. Napier and Dodd calculate a theoretical value for s by using a Monte Carlo simulation technique to look at random collisions between particles. Now collisions can cause erosion or fragmentation depending on the size of the incident particle. The authors find that erosion is negligible, a rocky asteroid typically only losing 8% of its mass before it suffers fragmentation. The collision fragments have an s value of between 1.5 and 2.0, the s value increasing with collision energy.

Starting with a sample of 7,400 bodies of differing mass and an s of 1.80, these authors found that 500 random collisions did not change s at all but that after this number the s value slowly increased reaching about 1.90 after 800 collisions. The larger bodies would not obey the power law because they were not being replenished. Napier and Dodd tried a second theoretical model where bodies of the same mass were allowed to collide and coalesce on collision. Independent of whether the accreted particles are replaced s quickly reaches a value of 1.5. These findings indicate that observed asteroidal s values can be produced by fragmentation and also by planetary disruption but not by accretion, thus removing one theory of origin.

Asteroids have rotation periods of around 8 h which for the larger ones is close to the limit for rotation-induced fission. Napier and Dodd find that the observed spin periods can be produced by fragmentation as there is abundant collisional energy available whereas accretion would give unobservably long periods. Fragments from a disrupted planet, however, would have rotation periods similar to that of the planet, which could quite easily have been in the region 8 to 10 h. So once again accretion is ruled out but the other two theories are tenable.

The authors have pointed out (*Nature* **242**, 250; 1973) that Ovenden's hypothetical planet cannot have been disrupted by the release of chemical or nuclear energy or by tidal instability induced by Jupiter. Disruption could be caused by detonation but chemical detonation is of too low an energy while nuclear fission detonation requires the sudden creation of a large supercritical mass within the planet. Another possibility is that the solid planetary mantle constrained a high pressure core until breaking point was reached; however, the tensile strength of rock is such that a planet of mass greater than 4×10^{23} g would not disperse the fragments to infinity and it would be impossible to lose the 99.9% of Ovenden's planet to leave just the asteroids remaining.

So only the planetoid hypothesis is left. This is consistent with meteorite geology which shows evidence of a cooling phase and shock processes which could have occurred at fragmentation. It is temporally reasonable as collision

dynamics indicate that Ceres would collide with one of the 30 asteroids capable of fragmenting it every 10^{10} yr indicating that the large asteroids are original planetesimals. More-

over, the dispersion of fragments after collision can produce orbits of reasonable inclination to the ecliptic just like those of the asteroids. D.W.H.

Cell movements in *Hydra*

CELL movement is a fundamental component of morphogenesis. Cells may move as three-dimensional masses, in sheets or as individuals. The movements must be controlled by signals available to the cells, and the signals may be involved in the control of development in general. One can therefore deduce much about developmental control from observing cell movements; for example, melanocyte migration in amphibians is under chemotactic control, as is the aggregation of slime mold amoebae.

In *Hydra*, Wolpert has shown that much morphogenesis is possible by a combination of cell movement and differentiation without any increase in cell number. Herlands and Bode (see *Nature*, **248**, 387; 1974) have now published observations on nematocyte migration, which confirm the general model for the control of *Hydra* development put forward by Wolpert and his collaborators.

Nematocytes are stinging cells used for the capture and paralysis of prey. They arise by differentiation from interstitial cells in *Hydra*'s body column, and migrate into the tentacles where they position themselves in the ectoderm. Although there are at least four types of nematocyte, Herlands and Bode examined the two commonest, desmonemes and stenoteles.

The authors grafted the upper halves of *Hydra* labelled with ^3H -thymidine to unlabelled lower halves, and also made the reverse grafts. They examined the originally unlabelled halves for labelled nematocytes. Nematocyte migration was strongly biased apically; indeed, basal migration only occurred towards buds. There was no migration into the peduncle.

As there was thus already a clear suggestion of apical

preference in nematocyte migration, the authors tried further grafting experiments to determine whether the signal was produced by the head or was some underlying property of the cells through which the nematocytes migrate. The authors labelled gastric regions of *H. attenuata* with ^3H -proline and grafted unlabelled heads to either the basal or apical ends. They then measured the rate of accumulation of labelled nematocytes in the unlabelled, grafted heads. They found that stenoteles accumulate at the same rate in basal and apical heads, but that desmonemes, for the first 3 days after grafting, accumulate in apical heads at a greater rate. After 3 days there was an increasing rate of migration by desmonemes into basal heads. These times correlate well with the times for polarity reversal by basally grafted heads found by earlier workers.

This interpretation was checked by grafting a head to the basal end and a peduncle to the apical end of a labelled gastric region. The addition of a peduncle is known to accelerate polarity reversal, and indeed there was no indication that the desmonemes migrated according to the original tissue polarity. Stenoteles, as before, migrated to the head regardless of polarity.

These results show that two kinds of signal are used—both an underlying property of the tissue, which may correspond to Wolpert's "positional value" and which can determine polarity, and a signal emanating from the head independent of polarity. This might be a chemotactic signal. The signals closely correspond to those already invoked for models of the control of *Hydra* development.

From a Correspondent

An SV40 that doesn't help adenovirus

THE inability of human adenoviruses to undergo a productive cycle of growth in simian cells—possibly because of the failure of a late function—can be overcome by coinfection with simian virus 40 (SV40). Adenovirus T-antigen (Feldman *et al.*, *J. Bact.*, **91**, 813; 1966; Malmgren *et al.*, *ibid.*, 262) and viral DNA are produced (Reich *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **55**, 336; 1966; Baum *et al.*, *Virology*, **34**, 373; 1966), whereas the production of some late protein is inhibited in monkey cells (Friedman *et al.*, *J. Virol.*, **5**, 586; 1970; Henry *et al.*, *Nature new Biol.*, **233**, 39; 1971). The region of SV40 DNA that specifies its helper function was located in an adeno-SV40-nondefective hybrid by Lewis and his colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **63**, 1128; *J. Virol.*, **11**, 655; 1971). These workers selected, from adenovirus populations that had been grown in the presence of SV40, mutants that were able to grow productively on monkey cells without the assistance of exogenous SV40 virus.

Some of these mutants contained segments of SV40 DNA covalently linked to adenovirus DNA sequences. In these adeno-SV40 hybrids, the region of the adenovirus substituted for by the SV40 sequences does not seem to be essential for adenovirus growth. The substitutions of SV40 sequences all begin at the same place in the adenovirus genome (14% from one end) but in different mutants the SV40 sequences can range in amount from 11–43% of the SV40 genome, the smallest being 17% of the genome (see *J. Virol.*, **12**, 643; 1973). All these SV40 sequences start from one place on the SV40 physical map which is 0.11 map units to one side of the *EcoR* cleavage site (see *J. Virol.*, **12**, 653;

1973). The exact nature of the SV40 helper function is not known but it has been mapped in a region of the genome that is thought to specify early functions.

Jerkofsky and Rapp tested the enhancing ability of some temperature sensitive mutants of SV40. Both late mutants, which did synthesise viral DNA, and an early mutant, *tsA7*, which did not synthesise viral DNA, but did induce host DNA synthesis (*J. Virol.*, **8**, 516; 1971), were able to enhance the production of adenovirus at the nonpermissive temperature in monkey cells. These results support their idea (*Virology*, **51**, 466; 1973) that an early function of SV40, possibly related to the induction of host DNA synthesis, is involved.

Kimura, in this issue of *Nature*, has tested the helping ability of temperature sensitive mutants of three complementation groups defined by Kimura and Dulbecco (*Virology*, **52**, 529; 1973). He also finds that late mutants will enhance adenovirus growth at the nonpermissive temperature. The early mutant *ts640* was unable to facilitate adenovirus growth at the nonpermissive temperature although enhancement was normal at the permissive temperature. This mutant *ts640*, like *tsA7*, is temperature sensitive for the production of viral DNA. It is not known whether *ts640* induces host DNA synthesis; it may differ in this respect and thus represent a second class of early mutants of SV40 (possibly a fourth complementation group). A further characterisation of *ts640* should be accomplished soon.

Kimura has mapped his genetic marker in the SV40 helper function: it occurs between 0.11 and 0.28 map units on the SV40 physical map. From a Correspondent

Wasa's cannon balls saved from corrosion

ON August 10, 1628 the Swedish warship *Wasa* started on her maiden voyage out of Stockholm harbour. She sank within minutes. 333 years later she was raised and is now a magnificent tourist attraction. She is also, to Mr L. Barkman of the Statens Sjöhistoriska Museum in Stockholm, "an enormous corrosion experiment". Barkman, along with Dr O. Arrhenius and Mr E. Sjöstrand report on the problems of preventing further rusting away of iron from *Wasa* in a recent publication of the Korrosionsinstitutet in Stockholm (*Conservation of Old Rusty Iron Objects*, Bulletin 61E, 1973; SKr 16.00).

Even at the time of the *Wasa* mishap the water in Stockholm harbour was fairly noxious. Dumping of rubbish had probably ensured that there was little or no dissolved oxygen present. The water was saline and at a temperature of around 3° C. Into this murky environment dropped a thousand iron objects such as guns, 24-pound cannon balls, chain shot and pike shot. Thousands of nails and one-inch diameter bolts also went down, but of these only two fragments remain. The guns were at first held down in their carriages, but were soon salvaged by the primitive means available in the seventeenth century. Presumably the forgings rusted rapidly—today there is practically no sign of them on the 64 gun carriages and 52 gun ports. The environment is still hostile. New one-inch diameter iron bolts were used during the salvage operation, and after a year the diameter of the worst affected had been reduced to one third of its original value.

While forgings such as nails, bolts and gun fittings disintegrated, cast iron objects fared much better; not that the corrosion was any the less, but castings (cannon balls, for instance) have retained their structure. This is believed to be an effect of the higher carbon (3½%) and silicon (1%) content of the cast-iron balls. Analyses of the rusty outer layer of a typical sample showed that the percentage of carbon and silicon in the corroded material was up to 12% and 5% respectively, whereas iron was down to 43%. Nearly all the iron was in oxides, but there was about 1% chlorine, mostly from chlorides formed in salty water. In a limited-oxygen environment the final product of the oxidation process would be Fe_3O_4 , black magnetite.

The rusted outer layer of cannon balls was on average 1.4 cm thick; this implies a lower corrosion rate than for the bolts, but the majority of balls were protected by mud. It is clear that in the corroded layer many of the iron compounds have been dissolved away but a

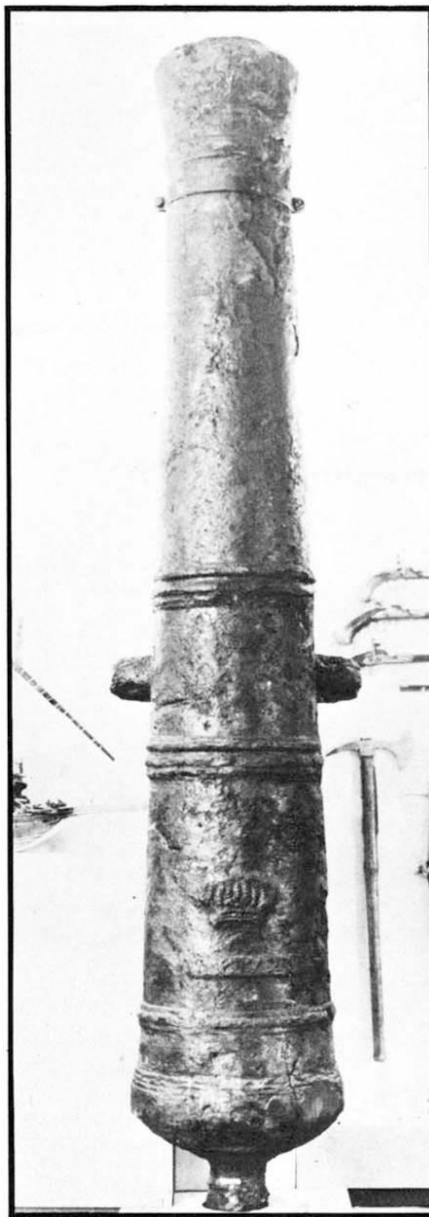
total collapse of the fabric of the rusted layer has probably been averted by the enriched carbon and silicon fraction.

The dissolved iron compounds did not get far, as there were no currents. When *Wasa* was raised it was a rich mixture of colours, from mud mixed with precipitated oxides and salts. Stalactites of iron oxides, silicates and sulphates hung from the decks. The much admired black oak colour of the hull was the result of impregnation with several tons of iron.

A very serious problem arose the moment the iron objects came out of the water. Oxidation continued—now in an oxygen-rich environment so the end product is Fe_2O_3 , red haematite, but more damaging was the chloride activity. In moist air, FeCl_2 and FeCl_3 go through a cyclic process, precipitating iron hydroxide. The pitting that this

causes is the most dangerous threat to iron that has been in seawater, and the threat cannot be arrested by standard cleaning and drying processes. Corrosion in the atmosphere would have rapidly destroyed the museum exhibits. Dramatic evidence of this was already present in Stockholm. A cannon from a seventeenth century wreck had been salvaged in 1953, scraped, brushed, treated with antirust oil and exhibited. The photographs show its decline in 18 years.

Within a month the *Wasa* specimens were visibly corroding further; one cannon-ball fell apart. Unlike most corrosion problems in which the aim is to clear the surface of rust, in this case it was necessary to halt the chemical processes but keep the structure intact. To do this the Stockholm workers decided to attempt to reduce the oxides and



Cannon from the *Riksäpplet* which foundered in 1676: left, after salvaging in 1953; right, the same with severe corrosion damage in 1972.

chlorides to the metallic form. Hartwich had first done this in the 1880s with small rusty samples at 500 °C in a hydrogen atmosphere; the process is widely used in industry, but not in museums.

A cylinder of depth 126 cm, radius 23 cm was loaded with the samples (for instance six cannon balls) which were then placed in a furnace in hydrogen. Reduction was carried out at 600 to 800 °C and after a period of about a day the process was complete—not only were oxides absent but chlorides too had been reduced to iron. Chemical analyses show that there was generally no trace of chlorine and all the iron was in the metallic form. Subsequent coating with an antirust agent and accelerated rusting tests show that the objects are now fully protected against corrosion. They now sit in the Wasa Museum—uncorroding and with the right shape.

Success of super labs at Cologne meeting

from a Correspondent

THE Spring meeting of the Cologne Institute of Genetics (February 22-24) opened with a symposium on tumour viruses which, as W. Doerfler (Cologne Institute of Genetics)—who organised it—pointed out, really dealt with DNA viruses, since even the RNA viruses discussed by P. Vogt (University of Southern California) and J. Dahlberg (University of Wisconsin) have a transitional DNA phase.

Transcriptional maps of the adenovirus genome in productive infection are emerging both from the Wallenberg Laboratory at Uppsala and from Cold Spring Harbor. L. Philipson (Wallenberg Laboratory) described the techniques used by his group to obtain adenovirus DNA fragments from cleavage with restriction enzyme and labelled either in the heavy or the light chain. The amount of labelled DNA could be measured after the templates had been hybridised with excess unlabelled RNA, to give the quantity of DNA transcribed from either the light or the heavy strand of the restriction enzyme fragments.

Philipson's results proved entirely compatible with those of the Cold Spring Harbor group, presented at the symposium by W. Keller. The highlights of Keller's talk were the description of a new approach to sequencing viral DNA using a series of restriction enzyme cleavages which should greatly facilitate what is normally a long and painful process, and the results of the analyses of six hamster cell lines transformed with adenoviruses.

All the transformed lines seem to

hand, at least a portion of restriction fragment F located near the middle of the DNA towards the C fragment end was absent from all the lines. Keller speculated that the adenovirus DNA was integrated into the host chromosomes and that the functions contained in the retained portion of fragment A were necessary for its maintenance.

A. Lewis (National Institutes of Health), in a thorough analysis of the non-defective adenovirus-SV40 hybrids, described the unequivocal mapping by heteroduplex analyses of the order of several early SV40 functions. The question whether viral DNA becomes integrated into 'transformed' lymphocytes was raised by T. Lindahl (Karolinska Institute), who showed that the link between Epstein-Barr virus DNA to lymphoblast DNA was not stable in alkali, although a substantial portion of the viral DNA banded in caesium chloride with host DNA. Only when the DNA was sheared into small fragments were the host and viral DNA sequences effectively separated by isopycnic banding.

Vogt effectively summarised the work on RNA tumour viruses with a lucid exposition of the dilemmas with which analyses of the structure of oncornavirus are fraught. Most of the data, including those on the kinetic complexity of the genome, suggest that it is haploid and segmented. But reservations on this conclusion must remain because all of the RNA segments, and not just one, decrease in size on the mutation of sarcoma viruses to become defective or leukaemogenic.

The symposium concluded with a *tour de force*: the announcement of the sequence of the transfer RNA molecule which serves as the primer for DNA synthesised on the 70S RNA template by reverse transcriptase. This was the fruit of a collaborative project involving Dahlberg at Madison (who presented the paper) and M. Bishop's laboratory at the University of California in San Francisco, and it illustrated one of the most conspicuous incidental points brought out by the meeting. This was the contrast in the accomplishments of the large institutionalised collaborative groups such as those at Uppsala, Cold Spring Harbor, NIH, and on the San Francisco-Madison axis, and those of the smaller laboratories, both European and American, represented at the meeting. It was the superlabs which, by approaching complex problems from several directions simultaneously, emerged as the more effective, despite the inevitable loss of scientific identity resulting from the dilution of credit among individual contributions.

have a portion of the terminal restriction fragment A and all but one contain at least a portion of the C fragment located at the other end. On the other

Perfusing the female reproductive system

from our

Steroid Biochemistry Correspondent

WITH the well known limitations of most *in vitro* techniques for studying the metabolism of tissues, the use of perfusion or superfusion techniques has become more popular. In the field of reproductive biology, interesting results have been obtained by perfusion of the human ovary, placenta or foeto-placental unit. Tojo *et al.* (*Amer. J. Obstet. Gynec.*, **118**, 119-129; 1974) have now achieved *in vitro* perfusion of the human uterus and fallopian tubes as well as the ovary.

Before removing the utero-tubal-ovarian unit, the arterial pressure, pulse rate and blood flow were measured and the pressure wave form of the uterine arteries was recorded. The unit was connected to the perfusing machine through the bilateral uterine arteries and the bilateral uterine and ovarian veins and was perfused in such a way that the pulse rate and pressure of the input of the perfusate were similar to those expected in the uterine artery *in vivo*. This was achieved by using a Bellofram-type artificial heart driven by a liquid amplifier, a pressure wave form regulator for the perfusate, an oxygenator, organ chamber, suction pump for venous return and an autoregulatory thermocontrol mechanism.

The viability of the unit during perfusion was tested by measuring the erythrocyte count, haematocrit, pO_2 , pCO_2 and pH values and the haemoglobin, lactate and pyruvate content of the perfusate.

Tojo *et al.* obtained successful perfusions of three utero-tubal-ovarian units, one obtained during the menstrual period and two during the mid-luteal phase of women with normal ovarian function. Addition of human chorionic gonadotrophin (HCG) to the perfusate increased the progesterone secretion from the ovary obtained during the luteal phase but not from that obtained during menstruation. When tissues obtained at the thirteenth week of pregnancy were perfused, there was a rapid increase in the HCG content and a gradual increase in the content of human placental lactogen (HPL) of the perfusate. Active nucleic acid synthesis was shown to occur in the trophoblastic tissue.

These preliminary results suggest that the utero-tubal-ovarian unit maintains its viability for some considerable time when perfused. If this is confirmed in further investigations, application of this method should help in the elucidation of many problems of reproductive physiology and pathology.

Rethinking about the sarcoplasmic reticulum

from a Correspondent

COINCIDENTALLY independent groups in London, Toronto and Heidelberg, have published in three different journals results that clarify the disposition and functions of membrane proteins in the sarcoplasmic reticulum of rabbit skeletal muscle.

Major proteins

The sarcoplasmic reticulum is a membrane specialised for transport and binding of calcium in muscle tissue and, as it has few proteins, it presents considerable advantages for study of cation transport through membranes. The major protein is a Ca^{2+} -activated ATPase (molecular weight approximately 102,000-115,000) which is tightly integrated into the membrane and is only released by detergents. Several other acidic proteins can be removed more easily, by extraction with chelating agents for instance. Calsequestrin, a protein with the ability to bind large numbers (30-40 per mole) of calcium ions at rather low affinity in the presence of 0.1M KCl, was originally suggested by MacLennan to play a part in cation binding in the reticulum. Calsequestrin is a glycoprotein and contains about 1 mol of sialic acid, clearly not enough to account for the total number of cation binding sites, most of which probably involve acidic amino acid residues of the protein.

Another acidic protein, with molecular weight 55,000, binds calcium ions with high affinity even at high ionic strength (0.1M KCl) but with low capacity (about 1 mol per mol). The other acidic proteins bind calcium but with very low affinity at any ionic strength. There is, however, some genetic variability in the total number of high or moderately high affinity calcium binding proteins and certain rabbits contain a second calsequestrin that is slightly smaller, with molecular weight 44,000 (MacLennan, *J. biol. Chem.*, **249**, 980-984; 1974). The mutation in this membrane protein seems to be a deletion and loss of a peptide sequence rich in methionine and cysteine. The mutant sarcoplasmic reticulum seems to be more active in Ca^{2+} transport than normal sarcoplasmic reticulum and the Ca^{2+} binding ability of the mutant protein is not affected by the deletion.

Calsequestrin

Calsequestrin was believed originally to be located in the interior of the sarcoplasmic reticulum and to play a part in sequestering calcium transported to

the inside of the vesicle by the Ca^{2+} -activated ATPase. Thorley-Lawson and Green (*Eur. J. Biochem.*, **40**, 403-413; 1973) suggest, however, that calsequestrin may be at the exterior face of the sarcoplasmic reticulum membranes, in sites exposed to the aqueous environment. This is certainly a more likely location for an extrinsic protein such as calsequestrin, which can be largely removed from intact closed membrane systems by extraction with 1 mM EDTA. The material removed by the chelating agent is reversibly bound to depleted membranes in the presence of Ca^{2+} indicating a binding to the lipid bilayer mediated by cations, with Ca^{2+} perhaps involved in some interaction between the acidic calsequestrin protein and the polar head groups of phospholipids. Candidates for a function as an internal calcium store may therefore have to be looked for elsewhere; possibly the acid protein of molecular weight 55,000 fulfils this role.

Calcium-activated ATPase

A second revision of the original model proposed by MacLennan for membrane proteins in the sarcoplasmic reticulum concerns Ca^{2+} -activated ATPase. These molecules were thought to be entirely represented by the 7.5-9 nm diameter particles seen in the freeze fractured faces of the membrane, that is to say, placing the ATPase molecules within the hydrophobic interior of the membrane. The recent reinterpretation makes the more acceptable proposition that the particles revealed in freeze fractured preparations represent the hydrophobic 'tail' of the ATPase that is firmly integrated into the membrane interior. The density of intramembranous protein domains is similar to that found in human erythrocyte membranes and representing the hydrophobic 'tail' of the major glycoprotein. About half of the Ca^{2+} -activated ATPase molecule is exposed on the exterior surface of the lipid bilayer where it is available for reaction with trypsin or lactoperoxidase (Thorley-Lawson and Green, *loc. cit.*; Migala, Agostini and Hasselbach, *Z. Naturf.*, **28**, 178-182; 1973; Stewart and MacLennan, *J. biol. Chem.*, **249**, 985-993; 1974). Since the sarcoplasmic reticulum is impermeable to protein reagents, trypsin and lactoperoxidase will initially only modify proteins on the external surface of these microsomes.

Structural relationships

The ATPase forms projections which consist of a 4 nm knob on a 2 nm stalk extending outwards from the external face of sarcoplasmic reticulum and seen in negatively stained preparations.

Very similar projections are produced in vesicles formed artificially from purified ATPase by removal of detergent from solubilised preparations of the enzyme. The projections are destroyed by high concentrations of trypsin with loss of enzymatic activity. ATP (and sucrose) protects the loss to some extent. Thorley-Lawson and Green (1973) found that at lower levels of trypsin, ATPase is split into two pieces of very similar size (40,000 and 55,000) which remain attached to the membranes. The fragments of molecular weight 60,000 are degraded to yet smaller units of molecular weights 33,000 (that contains the phosphorylated site) and 24,000, and all of these are iodinated by lactoperoxidase added externally suggesting that they exist on the outer surface of the membrane. The fragment of molecular weight 60,000 thus represents the stalk of the ATPase and the 55,000 molecular weight fragment, which is not iodinated, is buried within the membrane, as expected for a hydrophobic 'tail' equivalent to particles of the size seen in the freeze fractured faces of the internal membrane. The internal particles and the non-iodinated fragments of molecular weight 55,000 persist within the membrane of extensively trypsinised preparations which is consistent with these being the part of the ATPase integrated into the membrane. Thorley-Lawson and Green found that this part of the enzyme molecule is not iodinated even when both faces of the membrane are accessible to labelling reagents. If the ATPase does span the width of the membrane therefore, and this is the simplest model to explain the Ca^{2+} transfer across the membrane, it does not expose any significant amounts of iodinated tyrosine nor trypsin sensitive bonds in the region.

The structural relationship of the intramembranous particles and Ca^{2+} -activated ATPase is also implied by their coordinate increase in the muscle microsomes during chick embryonic development. Two papers by Martonosi and his colleagues (*J. biol. Chem.*, **249**, 612-623 and 624-633; 1974) describe developmental changes in the sarcoplasmic reticulum of chick skeletal muscle. The increased transport of Ca^{2+} during embryonic and postnatal development is directly related to concentration of Ca^{2+} -activated ATPase in the membranes of the sarcoplasmic reticulum. There seems to be an inverse relationship during embryogenesis between the appearance of the Ca^{2+} -activated ATPase and a Ca^{2+} -insensitive Mg^{2+} -activated ATPase. This second enzyme appears early in development and subsequently declines. It would be interesting to know whether these ATPases are independently controlled. The alternative is to assume a precursor

function for the Mg^{2+} -activated enzyme which acquires calcium sensitivity at a relatively late stage in development, for example, by interaction with 'coupling factors' which link ATP hydrolysis to Ca^{2+} transport. The lack of a subunit structure for the Ca^{2+} -activated ATPase of rabbit sarcoplasmic reticulum tends to make this assumption less likely, although it is an intriguing possibility.

Solar wind blows gusty at sunspot maximum

by our Cosmology Correspondent

THE continuing story of the influence of the Sun on terrestrial events has developed a stage further with the report by Intriligator of observations which provide the first evidence for long term variations in the solar wind associated with changes in the solar cycle (*Astrophys. J. Lett.*, **188**, L23; 1974). Events such as weather variations and modulation of the Earth's spin rate associated with the solar cycle of activity are now becoming well documented, and in recent years it has been established that there is a solar modulation of cosmic rays which is in anti-phase with the solar cycle. All this evidence points to variations in the solar wind as, if not the root cause, then at least the next link in the causal chain. But since suitable space vehicles which can provide essentially continuous monitoring of the solar wind have only been available for some ten years—about the length of one solar cycle—it is hardly surprising that such solar wind variations have only now been directly confirmed.

Intriligator's work is concerned with variations in the solar wind which are the result of changes in the high speed stream structure. The data were obtained by Pioneer and Vela spacecraft; they show variations in the number and intensity of high speed proton streams, and the average solar wind speed, between 1965 and 1971. Early in the period studied a high solar

wind speed of 626 km s^{-1} was measured, on January 20, 1966, and in all five high speed streams (the slowest with velocity 497 km s^{-1}) were observed between December 25, 1965 and February 3, 1966. But this represented the low point of activity during the period of the investigation.

When longer stretches of data are examined, it turns out that there were more high speed streams in 1968 (44) than in any of the other years of the study; as Intriligator puts it, the solar wind is more "gusty" around the time of maximum solar activity. On 230 days in 1968 there was high speed streaming activity in the solar wind; at the other extreme, all but 73 days out of 270 studied in 1971 were free from gusts.

This immediately offers an explanation of the modulation of cosmic rays over the solar cycle. At solar maximum, there is a much greater chance that cosmic rays entering the Solar System will encounter high speed streams, and this produces more modulation of the cosmic rays. When the solar wind is calm, it is easier for cosmic rays to penetrate the solar wind and reach Earth. The yearly average of the solar wind speed itself varies over the solar cycle, and is highest at solar maximum; there seems little doubt that this is a real effect which provides an important insight into the workings of the Solar System.

Genome organisation in eukaryotes

from a Correspondent

ANALYSIS of the organisation of nucleotide sequences in DNA of the more highly evolved eukaryotes is proceeding rapidly at present and has revealed some intriguing but so far unexplained phenomena. Following up their initial observations on the formation of DNA rings and other circular structures (*J. molec. Biol.*, **51**, 621; 1970), Thomas and his associates in a series of papers (*ibid.*, **77**, 25-99; 1973) have estimated that as much as half the DNA in mammals, insects and amphibians is composed of tandemly repeated or intermittent tandemly repeated sequences clustered in groups which they call *g* regions. The number of *g* regions in three *Drosophila* species is about 5,000 which corresponds to the number of cytologically observable bands or chromomeres in their polytene chromosomes.

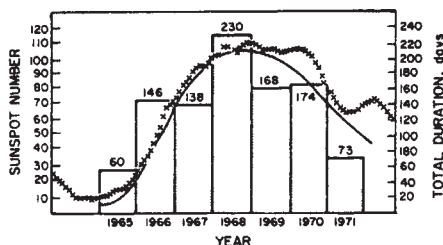
Similar measurements for the mouse and *Necturus*, which have respectively fifteen and seven hundred times more DNA than *Drosophila*, reveals that the number of *g* regions is about 40,000 and 1.5 million respectively. This probably means that the one to one correlation between *g* region and

chromomere number observed in *Drosophila* does not hold in other organisms since the chromomere number (lampbrush chromosome loops) in *Triturus* has been estimated at 4,000. These results have relevance for the problem of the very large differences in genome size between closely related organisms. Increased genome sizes seem to be based largely on increased numbers and only marginally on increased size of *g* regions, since the estimated sizes of *g* regions in mouse and *Necturus* are only twice that of *Drosophila*.

The kinds of experiments Thomas and his colleagues have done to reach these conclusions are the measurement of the frequency and contour length of rings and ringlike structures observed by electron microscopy in preparations of DNA renatured to low *Cot* values after treatment with *Escherichia coli* exonuclease III, an enzyme which digests one strand of a DNA duplex from 3' to 5'. DNA molecules resected by this enzyme will have two single strand ends which if complementary will tend to renature to give rings and other ring-like structures. No rings are produced by treatment of bacterial DNA in the same way, though many phages may cyclise through the presence of so called sticky ends which may occur naturally as in λ or produced by exonuclease III treatment as in T7.

Thomas *et al.* have shown that the frequency of rings is linearly related to the extent of resection up to a maximum plateau level which is 300 nucleotides for mouse and *Drosophila* and 500 for *Necturus*. These figures are presumably related to the length of the repeating sequences involved. Providing rings are formed in optimal resection and renaturation conditions, the frequency of rings is primarily a function of DNA size and is maximum with DNA of 1-2 μm in length regardless of organism. Such rings have very good thermal stability suggesting that the region of overlap is of the order of 200 nucleotides with very little if any mismatching, since thermal stability under ionic conditions reflects both length and fidelity of hybrid duplex. This means that the minimum length of the repeat unit is 200 nucleotides and that they show very little divergence by base substitution.

Fewer rings are created both with larger and with smaller DNA molecules. The reduction with increasing length is interpreted to mean that related repeated sequences are clustered into regions which Thomas *et al.* call *g* regions. An alternative explanation for this observation—that longer fragments have a greater distance separating potentially reactive single strand ends resulting in slower renaturation kinetics—was eliminated by a study of the effect of fragment length on the rate of



Total number of days on which high speed "gusts" were observed in the solar wind. —, Smoothed sunspot numbers; —x—, measured sunspot numbers. Data for 1965 and 1971 are based on 136 and 270 days of data, respectively.

cyclisation. Similarly the reduction with small DNA molecules was not due to slower renaturation kinetics of small fragments resulting from their decreased flexibility. It is argued that reduced ring frequency with molecules less than 1.2 μ m is evidence for tandem repeats being spaced by non-repetitious sequences. Although Bick, Huang and Thomas (*J. molec. Biol.*, **77**, 75; 1973) tried to find evidence for the presence of this predicted non-repetitious DNA by examining rings prepared for electron microscopy in high concentrations of formamide, which would reveal unpaired single stranded regions within the terminal region of overlap, they were unable to do so. They concluded that either the non-repetitious sequences do not exist, which is unlikely, or they are too long or too short to be observed.

Similar conclusions concerning the interspersion of repetitive and non-repetitive sequences have been reached by other groups using different techniques. For instance, Davidson *et al.* (*J. molec. Biol.*, **77**, 1-23; 1973) used labelled *Xenopus* DNA sheared to various known lengths denatured and then renatured, in the presence of excess cold *Xenopus* DNA of 450 nucleotides length, to C_{ot} 50, at which point predominantly repetitive sequences have renatured. They found that as the length of labelled DNA increases so a greater amount is bound to hydroxyapatite. Hydroxyapatite binds double stranded but not single stranded DNA at low salt concentrations and therefore allows their separation. In the conditions of the experiment any non-repetitive sequences, although remaining single stranded, will bind to hydroxyapatite if they are covalently linked to renatured repetitive sequences.

The curve relating the proportion of ^3H -labelled DNA fragments containing a repetitive element to fragment length shows three things. A steep linear increase (4% per 100 nucleotides) up to an inflexion point at about 700 nucleotides length and 60% fragments bound, followed by a slower linear increase (0.7% per 100 nucleotides) up to 4,000 nucleotides length and 80% fragments bound. The first part of the curve does not extrapolate through zero bound at zero length but through about 20% representing the amount of repetitive DNA in the genome. Davidson *et al.* interpret these data on the basis of a model in which 50% of the *Xenopus* genome consists of repetitive elements about 300 nucleotides long interspersed with non-repetitive sequences 700-900 nucleotides long. A further 25% consists of repetitive sequences interspersed with longer (>4,000 nucleotides) non-repetitive sequences. The remainder is composed of unique sequences (20%) without detectable repetitive elements and of tandem, low complexity repeti-

tive sequences (5%) without detectable non-repetitive elements. Similar experiments with sea urchin DNA (Graham *et al.*, *Cell*, **1**, 127; 1974) show that an identical model may be constructed. Furthermore, in two earlier papers (Kram *et al.*, *J. molec. Biol.*, **64**, 103; 1972; Wu *et al.*, *ibid.*, **64**, 211; 1972) on the organisation of repetitive sequences in *Drosophila* DNA other groups reached similar conclusions.

Although it seems likely on present evidence that a general model in which repeated sequences alternate with unique sequences in eukaryotic DNA can be constructed, a good deal more work is required to reveal in detail which repeating patterns are universal and to provide some notion of their relevance to genome function.

Pattern formation in insects

from our *Insect Physiology Correspondent*

THERE are two ways in which the differentiated pattern of the living body may be supposed to arise. Each group of cells forming a given element in the body pattern may be derived from one progenitor cell, so that each component of the pattern represents a clone of cells which continues to propagate a persisting state of determination. Or, the cells forming an element of the body pattern may not have a different ancestry, or 'lineage', from the surrounding cells: their characters may have been determined by their position in the body. It can, of course, be argued that the second alternative is merely a repetition later in development of the same process by which the initial clones of the former alternative came into existence.

In normal development it seems clear that both these modes are concerned in the final generation of pattern. Many years ago, Sturtevant utilised the tendency of certain strains of *Drosophila* to form sexual mosaics (as the result of a spontaneous elimination of an X chromosome from somatic cells during embryonic development) in order to demonstrate the stage of development at which this mutation occurs and to relate this with the mosaic pattern in the resulting adult. This was done by coupling the mosaic strain with bristle characters that could be observed in the cuticle surface, and using the results to study the derivation or 'cell lineage' of the surface of the adult fly. Sturtevant found that related cells remain adjacent in patches without intermingling; that the occasional loss of an X chromosome happens at the early cleavage divisions; and that the mosaic patches of the opposite sex represent clones of these mutant cells.

Recently there has been a renewal of

interest in this approach to the problem of differentiation. Garcia-Bellido *et al.* (*Nature new Biol.*, **245**, 251; 1973) used somatic crossing-over induced by X rays to generate marked clones in *Drosophila* in which he could follow the distribution of characters in the cuticle of the wing surface. The resultant wings were made up of mixed clones, some of marked cells homozygous for the wild type allele of minute, M^+/M^+ ; others, the unmarked cells of the heterozygous strain, M^+/M . The marked cells have a much higher growth rate than the unmarked heterozygote. But in spite of this they did not overgrow the unmarked cells. It turned out that the wing is subdivided into eight compartments, and, provided a clone is generated after the time of final subdivision, it is strictly confined to one compartment. But within this compartment the cells could be differentiated by local control so as to contribute to the ordinary wing surface, to parts of wing veins, or to marginal bristles—depending not on cell lineage but on position at the time of differentiation.

Lawrence (*J. Embryol. exp. Morph.*, **30**, 681; 1973) now describes experiments in this same general field carried out on the milkweed bug *Oncopeltus*. He deals solely with the integument of the abdomen. Mild X irradiation of the egg led to somatic mutations which resulted in changes in the pterin pigments of the epidermal cells from the normal orange to deep orange, transparent, white or pink. As in Sturtevant's experiments these mutations resulted in clonal patterns of epidermal cells which are readily recognised by their unusual pigmentation. The results provide much information about the timing of determination for the structure of the abdomen, the number of cells which furnish the ancestry for a single abdominal segment and so forth. Again it is evident that the clones are subject to strict control. If the ancestral cell mutates after segmentation of the presumptive abdomen has been determined (during blastoderm formation), the clones are restricted to a single quadrant of a segment (dorsal or ventral, left or right). The daughter cells forming the members of a clone tend to hold together in a clump, but they can mix with their neighbours and the groups become fractionated to some extent. On the other hand, they cannot cross the boundaries between segments, between dorsal and ventral, or across the midline of the body.

The effective boundaries between segments are established at the time, 10-24 h after egg laying, when the limiting membranes of the cells are developing in the blastoderm. The nature of these and other invisible boundaries between compartments will be a key question in the understanding of differentiation.

Palaeomagnetic results and late Precambrian glaciations

M. W. McElhinny, J. W. Giddings & B. J. J. Embleton

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A combined late Precambrian to early Palaeozoic polar wander path, which differs from that previously proposed for Africa and South America, is derived for Gondwanaland. It suggests that Gondwanaland existed at least 750 Myr ago. The widespread distribution of late Precambrian glaciations can be explained in terms of this polar migration. It is predicted that the late Precambrian and Cambrian part of the North American polar wander path may be more complicated than previously supposed.

PIPER *et al.*¹ have tested whether the concepts of modern plate tectonic theory, as applied to several Phanerozoic orogenic belts²⁻⁶, can be applied to all ancient orogenic belts. Their analysis shows that all palaeomagnetic pole positions for Africa between 2,200 Myr ago and about 450 Myr ago (close of the Ordovician) lie on a single apparent polar wander path, irrespective of cratonic region. This suggests that the major cratonic areas were approximately in their present relative positions and orientations as early as 2,200 Myr ago. The younger intervening orogenic belts did not, therefore, result from plate accretion and subduction processes during an episode of major ocean closure which culminated in a continent-continent collision^{7,8}. Rather, the data support the view that these belts formed *in situ*, the stable cratonic nuclei remaining in place while the intervening belts themselves were being reactivated^{9,10}. Present data cannot, however, preclude the possibility that the belts resulted from the successive opening and closing of small intercratonic oceans.

Comparing with American Precambrian data, Piper *et al.*¹ further suggest that South America and, until about 1,000 Myr ago, North America may have been joined to Africa, and the concentration of all continental crust in one large mass until that time remains as a possibility. A further consequence of their interpretation is that if any of the Precambrian glaciations which affected south-west, central and north-west Africa, and parts of South America, originated between 900 and about 650 Myr ago, then they were formed in low latitudes^{11,12}.

New results from late Precambrian and early Palaeozoic formations in Australia (ref. 13 and J. W. Giddings and B. J. J. Embleton, unpublished information) and India^{14,15} allow us to extend the comparisons of Piper *et al.*¹ to include the whole of Gondwanaland. Our results lead to a different interpretation of the late Precambrian to early Palaeozoic apparent polar wander path. A consequence of this is that late Precambrian glaciations are restricted to high latitudes, as may be expected. Our interpretation does not, however, affect the general conclusions of Piper *et al.*¹, but leads to some predictions concerning the apparent polar wander path for North America.

Gondwanic palaeomagnetism

Palaeozoic and Mesozoic palaeomagnetic results, when referred to the reconstruction of Smith and Hallam¹⁶, suggest

that a common apparent polar wander path can be applied to the supercontinent from some time in the early Palaeozoic until its break up in the Mesozoic¹⁷. Interpretation of the early Palaeozoic section of the path has been uncertain because the poles tend to form rather a loose group in the region off north-west Africa. Stratigraphic sequences have been studied in both northern Australia and the Amadeus Basin of central Australia^{13,18}. The studied strata range in age from Precambrian-Cambrian through to the Ordovician. In each of these unrelated sequences a very large polar shift of 50°–60° of arc is observed during the earliest Cambrian, and in the Amadeus Basin¹⁸ the total shift during the early-Palaeozoic is about 90°. Figure 1 compares the Australian results on the reconstruction of Gondwanaland, with the corresponding late Precambrian and early Palaeozoic path for Africa and South America¹. It can be seen that the two Cambrian sections of the curves do not agree, and in fact have polar shifts in opposite directions.

There are two possible explanations of this disagreement. First, is that the earliest Cambrian sections of the two paths could refer to separate plates which collided to form Gond-

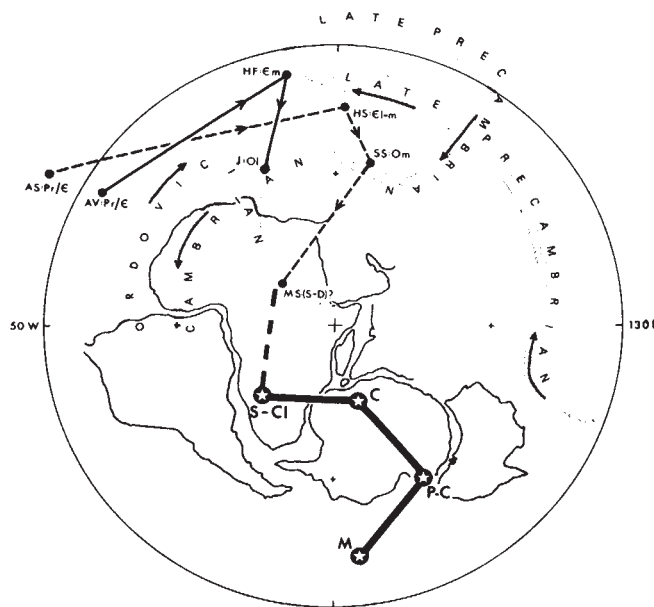


FIG. 1 Late Precambrian to early Palaeozoic apparent polar wander path for Africa and South America suggested by Piper *et al.*¹ (stippled path), plotted against the reconstruction of Smith and Hallam¹⁶ with Africa in its present day position. The large Cambrian polar shift observed in two stratigraphic sequences in northern Australia (solid line) and central Australia (dashed line) are shown for comparison. The pole designations are as listed in Table 1. Note that the path of Piper *et al.* places Africa in equatorial latitudes during the latest Precambrian. The solid black line connecting the mean Silurian-Lower Carboniferous (S-Cl), Carboniferous (C), Permo-Carboniferous (P-C) and Mesozoic (M) poles is the common path for this time interval^{13,17}.

TABLE 1 Gondwanic continents: late Precambrian-early Palaeozoic pole positions

Rock unit	Symbol	Age (Myr)	Palaeomagnetic Pole coordinates	
Africa				
Bukoban Sandstone	BS	Pr(~ 1000)	40°N	317°E
Kigonero Flags	KF	Pr(> 890)	12°N	273°E
Kleinkaras Dykes	KK	Pr(878 ± 41)	20°N	294°E
Gagwe Lavas	G	Pr(813 ± 30)	29°S	293°E
Bukoban Intrusives	BI	Pr(806 ± 30)	11°S	281°E
Mbozi Complex	M	Pr(743 ± 30)	72°S	248°E
Lower Buanji Series	LB	($< 1,350$)	87°S	83°E
Pre-Nama Dykes	ND	Pr(653 ± 70)	85°S	48°E
Plateau Series, Zambia (i)	PZA	($< 1,000$)	71°S	353°E
Plateau Series, Zambia (ii)	PZB	($< 1,000$)	60°N	25°E
Sijarira Group	SG	Pr/€	2°N	352°E
Ntonya Ring Structure	N	Pr(630 ± 24)	28°N	345°E
Klipheuvelformation	K	Pr/€1	16°N	316°E
Ouarzazate Volcanics	OV	Pr/€	30°N	237°E
Amouslek Tuffs	AT	€1	41°N	250°E
Sabaloka Ring Structure	SR	(> 540)	83°N	339°E
Fish River Series	FR	€1	55°S	317°E
Moroccan Lavas	ML	€m	53°N	34°E
Table Mountain Series	TM	0	50°N	349°E
Hook Intrusives	HI	01(500 ± 17)	14°N	336°E
Plateau Series, Zambia (iii)	PZC	Lr.Pal	10°S	352°E
Plateau Series, Zambia (iv)	PZD	Lr.Pal	22°N	19°E
Antarctica				
Charnockites	C	€u-01	2°S	20°E
Sør Rondane Intrusives	SRI	01-m(485 ± 25)	28°S	10°E
Australia				
Precambrian Dykes, B Group	B	Pr(750)*	24°S	282°E
Pound Quartzite	PQ	Latest Pr	60°S	6°E
Antrim Plateau Volcanics	AV	Pr/€1	9°S	340°E
Arumbera Sandstone	AS	Pr/€1	8°N	325°E
Aroona Dam Sediments	AD	$> €1$	36°S	33°E
Hugh River Shale	HS	€1-m	11°N	37°E
Hudson Formation	HF	€m	18°N	19°E
Lower Lake Frome Group (Flinders Ranges)	FRS	€m	8°S	25°E
Upper Lake Frome Group (Balcoracana Creek)	BC	€u	38°S	26°E
Jinduckin Formation	J	01	13°S	25°E
Stairway Sandstone	SS	Om	2°S	50°E
Mereenie Sandstone	MS	(S-D)?	41°S	40°E
India				
Malani Rhyolites	MR	Pr(745 ± 10)	42°S	115°E
Bhander Sandstone	BH	Pr/€	49°S	33°E
Upper Rewa Sandstone	UR	Pr/€	35°S	42°E
Upper Bhander Sandstone	UB	Pr/€	32°S	19°E
Purple Sandstone	PS	€1	28°S	32°E
Salt Pseudomorph Beds	SP	€m	27°S	33°E
South America				
Purmamarca Village	PV	€	61°N	293°E
South Tilcara	ST	€	52°N	27°E
North Tilcara	NT	€	49°N	24°E
Purmamarca	P	€	5°N	39°E
Abra de Cajas	AC	€	2°N	28°E
Salta and Jujuy	SJ	€-0	12°N	329°E
Salta	S	0	31°N	13°E
Sediments, Bolivia	SB	0	4°N	302°E
Urucum Formation	UF	0-S	17°N	347°E

Age symbols: *provisional Rb-Sr age; Pr, Precambrian; €, Cambrian; 0, Ordovician; S, Silurian; D, Devonian. Upper, Middle and Lower divisions denoted by u, m and l respectively.

References: Africa^{1,17,21}; Antarctica¹⁷; Australia¹⁸, McElhinny, M. W., Giddings, J. W., and Embleton, B. J. J., not yet published; India^{14,15,17,19}; South America^{17,20}.

wanaland during the mid-Cambrian. The timing then corresponds to the peak of the 550 ± 100 Myr pan-African orogeny, which was not the result of plate convergence¹. It thus seems unlikely that any one of these orogenic belts, such as the Mozambique belt, represents the suture between an eastern and a western Gondwanaland. The alternative is that it may be possible to re-interpret the African and South American data to conform with the Australian results.

Pole path for Gondwanaland

Table 1 lists all the palaeomagnetic poles for the Gondwanic continents between about 1,000 Myr and about 450 Myr (close of the Ordovician).

All the palaeomagnetic poles of Table 1 have been analysed with respect to the reconstruction of Gondwanaland of Smith

and Hallam¹⁶, and are shown in Fig. 2. We have been able to interpret all the data to account for the Cambrian polar shift observed in the stratigraphic sequences in Australia. All the South American poles designated Cambrian by Thompson²⁰ now fall on the Cambrian section of our combined path. African poles younger than about 700 Myr, but pre-Ordovician, have very poor age control, with the single exception of the 630 ± 24 Myr Ntonya Ring Structure of Malawi. We have been able to incorporate these poles into our combined path by minor rearrangement of their sequence without violating the tolerances on their age control. Significantly, this places the Ntonya pole at a point on the path just before the Precambrian-Cambrian boundary, whereas in the African path proposed by Piper *et al.*¹ it was out of sequence and inferred to have a much younger age.

We have not drawn the path through the two late-

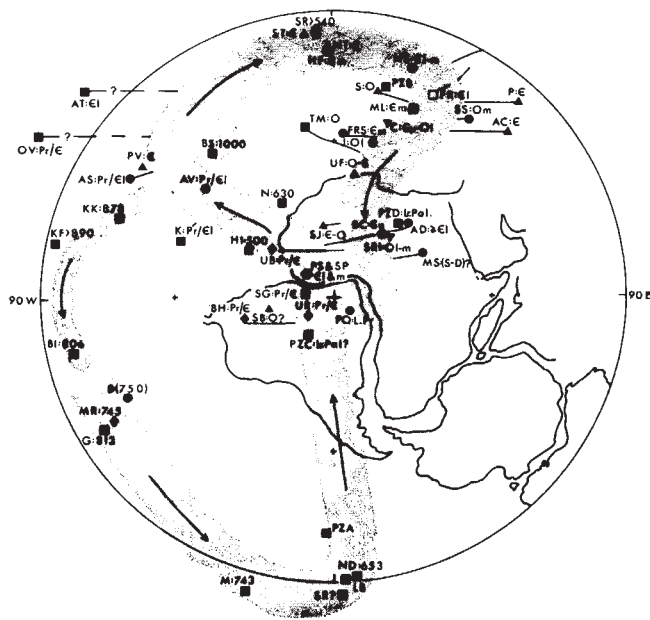


Fig. 2 Revised late Precambrian to early Palaeozoic polar wander path using all the Gondwanic poles listed in Table 1. Bearing in mind the uncertainties in some of the ages and the errors associated with the pole positions, all poles younger than 750–800 Myr are consistent with a common polar wander path when referred to the Smith–Hallam reconstruction¹⁶. The path is indicated by the broad band drawn through the poles. Before 750–800 Myr the sequence of poles is as yet only defined by African data indicated by the narrower band going back to about 1,000 Myr. ■, Africa; ●, Australia; ◆, India; ▲, S. America. Labelling as in Table 1.

Precambrian and early Cambrian Moroccan poles²¹ because this part of Africa might be a remnant of a Precambrian North America. It would not, however, be difficult to incorporate these into a slightly modified path, and then also identify a large Cambrian shift for North Africa to the pole for the Middle Cambrian Moroccan lavas. Latest Precambrian poles cluster in the region west or south of north-west Africa. The pole from the Cambro–Ordovician Hook Intrusives of Zambia is in this group but its circle of confidence is large enough for it to be associated with the later part of the curve in the region of the Sahara. The Cambrian poles from the Salt Range also seem to be out of sequence. This supports the suggestion of a separate Indus sub-plate¹⁵, but for an entirely different reason to that proposed. Originally the proximity of the Salt Range poles to those from the Bhandar and Rewa Series seemed to suggest that they were all of the same age¹⁴. A more likely late-Precambrian age for the Bhandar and Rewa Series now supposes that the structural setting of the Salt Range has caused their poles to be displaced from the Cambrian part of the curve. The pole from the Bolivian sediments of supposed Ordovician age falls in a region which suggests that these sediments may be rather older than supposed.

If the African part of the apparent polar wander path is to be extended back from the late Precambrian group it should continue southwards (Fig. 2) to the vicinity of the Mbozi Complex pole. It is at this point that our interpretation differs significantly from that of Piper *et al.*¹. The early Palaeozoic section of the pole path, as we have drawn it, represents the South Pole path (Fig. 2), but our interpretation obliges us to join up with what Piper *et al.*¹ regard as the North Pole path for about 700 Myr and older (Fig. 4 in ref. 1).

Unity of Gondwanaland

The 745 Myr Malani rhyolite pole for India plots close to a new Australian pole (J. W. Giddings, and A. R. Crawford, unpublished information) for which a provisional Rb–Sr age of 750 Myr has been estimated. These poles fall on the African curve near poles dated at around 800 Myr. This suggests to us that the consistency of the results for the Smith–Hallam reconstruction¹⁶ defines a single pole path extending back at least 750 Myr. It confirms the conclusion that the Pan-African orogenic belts must have been formed *in situ*¹. We can now extend the case of the intercratonic belts within Africa to those within Gondwanaland, such as the Mozambique belt and the eastern Ghats belt.

Late Precambrian glaciations

The occurrence of glacial deposits (tillites) in many late Precambrian formations throughout the world has led to the hypothesis that a widespread glaciation occurred just before Cambrian times^{22,23}. An essential point relating to this hypothesis is whether these glaciations occurred successively throughout polar migration or whether they occurred simultaneously and therefore perhaps extended to low latitudes. The interpretation of the late Precambrian to early Palaeozoic apparent polar wander path for Africa is crucial in this context because many of the late Precambrian glacial deposits are located in that continent. The pole path deduced by Piper¹¹ and Piper *et al.*¹ implies that the glaciated regions essentially occupied low latitudes, and that the glaciation was widespread¹². Our interpretation of the late-Precambrian to Cambrian pole path leads, however, to the conclusion that the pole migrated across South America (which was then joined to Africa) from south to north, during the late Precambrian. This is consistent with the occurrence of glacial deposits which are successively younger northwards along western Africa (Fig. 3).

In South West Africa two glacial episodes are recorded²⁴. Glacial horizons occur in the upper part of both the Damara

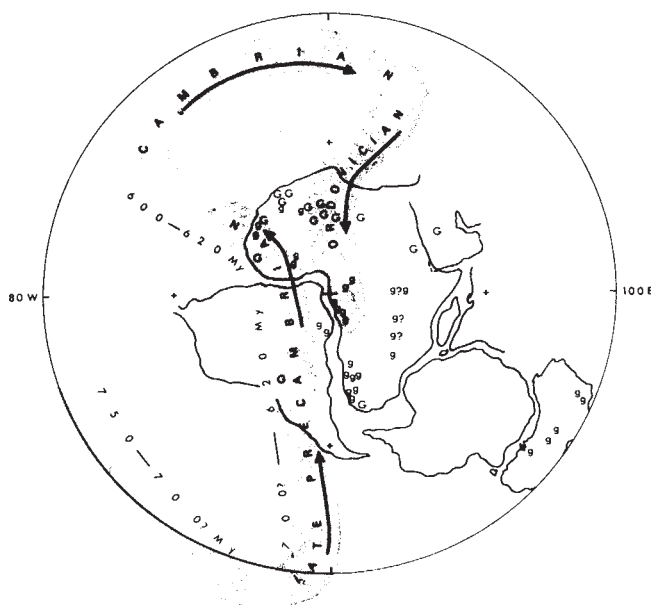


Fig. 3 The revised late Precambrian to early Palaeozoic apparent polar wander path for Gondwanaland compared with the distribution of glacial tillites. G.g. represent Ordovician and late Precambrian occurrences respectively. Glacial deposits in Africa possibly older than about 700 Myr and therefore not related to the drawn path have question marks. Successively younger late Precambrian occurrences along the west coast of Africa are matched by the south–north migration of the pole.

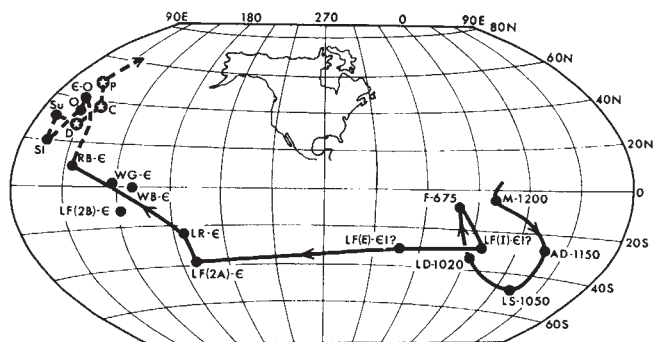


FIG. 4 Suggested revised North Pole apparent polar wander path for North America, which joins the Great Logan Loop³⁵ (1,200–1,020 Myr) to the Ordovician (O) part of the curve by means of a large Cambrian shift. Cambrian poles from: RB, the Ratcliffe-Brook formation¹⁸; WG, Wichita granites¹⁸; WB, Wilberns formation³⁹; LR, Lodore formation¹⁸; LF-I, 2A, 2B and E, Lamotte formation³⁸. Anchor points for the Great Logan Loop³⁵ are shown together with the Franklin pole³⁵ (F). Post-Cambrian Palaeozoic poles³⁹ are joined by a dashed line, the stars representing mean Devonian (D), Carboniferous (C) and Permian (P) poles.

and Nama Systems which are correlated with one another, and whose lower boundaries are not older than 650 Myr. Immediately underlying the Nama System are the Blaubecker Formation glacials and the Buschmannsklippe tillite. These are correlated with glacials of the Numees Formation which forms the upper part of the Gariiep Group. A felsite lava in the Kapok Formation, which corresponds to the lower part of the Gariiep Group, has an age of 720 Myr. The Blaubecker-Buschmannsklippe-Numees glacials are therefore very likely to be younger than 700 Myr. In Katanga the Pétit Conglomerate is probably of glacial origin and has a minimum age of 620 Myr (ref. 25). The Grand Conglomerate of Zaire is, however, probably older than 850 Myr (ref. 25) and therefore relates to a very much earlier glacial episode. In north-western Africa the Saharan 'Eocambrian' tillite has been dated at 620–650 Myr (ref. 26), whereas, in Ghana the Oti tillite has an age of 620 Myr (ref. 29). This succession strongly suggests that the centres of glaciation followed the path of the pole in late Precambrian times as it swept from south to north, past southern, western and north-western Africa, between about 700 Myr ago and 620 Myr ago (Fig. 3). It seems significant that the best dated African late-Precambrian pole, the 630 ± 24 Myr Ntonya result, plots just off north-west Africa (Fig. 2) corresponding to the time when this region was extensively glaciated.

During the Cambrian the broad trends of climatic change are perceptible²⁸. In North Africa the occurrence of thick successions of Lower Cambrian warm water deposits include also archaeocyathid reefs. Cambrian rocks are frequently brightly coloured, indicating a warm climate, but they give way to more drab Ordovician sequences which culminate in the great, late Ordovician, Saharan glaciation²⁹. These broad trends are not only compatible with the Cambrian and Ordovician sections of the pole path, but also resolve a previously puzzling palaeoclimatic anomaly³⁰. From near polar conditions in north-west Africa the broad loop taken by the pole during the Cambrian placed North Africa in low latitudes, with a return to intermediate, and finally polar, latitudes during the Ordovician.

It is not clear how the pattern of late Precambrian glaciation in Australia fits into the polar migration proposed in Figs 2 and 3. Dunn *et al.*³¹ cite ages of 750 Myr for the Sturtian-Moonlight Valley glaciation, but these are all based upon Rb-Sr measurements on shales. The interpretation of isochrons drawn through such data in terms of age of deposition is very much an open question (W. Compston, personal communication), so that the relationship of these ages to

those on our pole path is unclear. We make the general observation, however, that between 750 Myr and 600 Myr a polar migration of at least 180° is proposed with respect to Gondwanaland. Very large polar shifts are observed in almost all continents during the late-Precambrian and Cambrian. It is therefore not surprising that the poles migrated quite rapidly over different parts of the globe leaving records of glaciated regions as they passed over the various continents. In these circumstances it is not necessary to invoke the hypothesis of an extensive synchronous world-wide glaciation that extended to low latitudes (see also ref. 32). The decisive test between the two hypotheses will come from palaeomagnetic measurements on the actual glacial horizons in various places around the world. If these measurements show that many of these deposits were formed in low latitudes then this will support the hypothesis of a synchronous world-wide glaciation.

Great Logan Loop of North America

There is an impressive correspondence between an extended loop in the African apparent polar wander path and the so-called Great Logan Loop³³ of the North American path^{1,11}. Both of these loops cover the time range 1,200–1,000 Myr. Our interpretation of the African polar wander path means that the poles in this time range are now south poles which can only be matched with the presumed North American north poles for the same time. The fit of the two loops is impressive, and so we tentatively suggest that the late Precambrian to early Palaeozoic section of the North American polar wander path may be much more complicated than previously supposed. Irving and Park³⁴ have already noted the very tentative nature of this section of the North American curve. There is only a single pole (the Franklin pole, 675 Myr) between the end of the Logan loop at about 1,000 Myr, and the Cambrian. During the same period in Africa the polar shift was more than 180° (Fig. 2). During the Cambrian alone the pole shift was about 90° . Furthermore, Cambrian results from the Bohemian massif have previously been interpreted in terms of a large polar shift of about 130° (ref. 35) and a large polar shift is also recorded in the Cambrian of Siberia¹⁷.

The Lamotte formation of Missouri (Cambrian in age, although the fossil evidence is poor) shows four distinct palaeomagnetic poles³⁶. Poles from the higher horizons are in two groups (2A and 2B), related to their stratigraphical positions, and suggest a polar migration during the Late(?) Cambrian. Poles from the lower horizons seem to be anomalous. No reason is apparent for group 1 and the other (Locality E) is close to the Carboniferous pole for North America. Carboniferous remagnetisation has been proposed, although no evidence has been given either to suggest how this arose or why it occurred selectively at only one locality. Studies of the Ordovician Trenton Limestone³⁷ have shown that the hypothesis of widespread late-Palaeozoic remagnetisation of Lower Palaeozoic rocks is no longer tenable. We therefore interpret these poles as representing a polar migration during the Cambrian.

Figure 4 shows how it is possible to join the later Palaeozoic poles for North America through an extended Cambrian path, to the opposite poles of the Logan Loop. These poles become the north pole path for North America with the consequence that the plot of the Logan Loop in the Pacific^{33,34} represents the south pole path. It is then readily matched with the African loop (see ref. 1). Our prediction therefore is that extensive studies of late Precambrian and Cambrian rocks of North America (1,000–500 Myr) will reveal a complicated apparent polar wander path with a polar shift through at least 180° .

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- ¹ Piper, J. D. A., Briden, J. C., and Lomax, K., *Nature*, **245**, 244 (1973).
- ² Dewey, J. F., and Bird, J. M., *J. geophys. Res.*, **75**, 2625 (1970).
- ³ Dewey, J. F., *Nature*, **222**, 124 (1969).
- ⁴ Bird, J. M., and Dewey, J. F., *Bull. geol. Soc. Amer.*, **81**, 1031 (1970).
- ⁵ Hamilton, W., *Bull. geol. Soc. Amer.*, **81**, 2553 (1970).
- ⁶ Oversby, B., *Nature phys. Sci.*, **234**, 45 (1971).
- ⁷ Hurley, B. M., *Earth planet. Sci. Lett.*, **15**, 305 (1972).
- ⁸ Burke, K. C., and Dewey, J. F., in *African Geology*, 583 (University of Ibadan, Nigeria, 1972).
- ⁹ Clifford, T. N., in *Radiometric Dating for Geologists*, 299 (Interscience, New York, 1968).
- ¹⁰ Shackleton, R. M., in *Time and Place in Orogeny*, 1 (Geological Society, London, 1969).
- ¹¹ Piper, J. D. A., *Geophys. J. R. astr. Soc.* (in the press).
- ¹² Piper, J. D. A., *Nature*, **244**, 342 (1973).
- ¹³ McElhinny, M. W., and Embleton, B. J. J., *Tectonophysics* (in the press).
- ¹⁴ Athavale, R. N., Hansraj, A., and Verma, R. K., *Geophys. J. R. astr. Soc.*, **28**, 499 (1972).
- ¹⁵ Klootwijk, C. T., *Tectonophysics*, **18**, 123 (1973).

- ¹⁶ Smith, A. G., and Hallam, A., *Nature*, **225**, 139 (1970).
- ¹⁷ McElhinny, M. W., *Palaeomagnetism and Plate Tectonics* (Cambridge University Press, Cambridge, 1973).
- ¹⁸ Embleton, B. J. J., *Earth planet. Sci. Lett.*, **17**, 217 (1972).
- ¹⁹ Wensink, H., *Earth planet. Sci. Lett.*, **16**, 189 (1972).
- ²⁰ Thompson, R., *Earth planet. Sci. Lett.*, **18**, 266 (1973).
- ²¹ Hailwood, E. A., and Tarling, D. H., in *Implications of Continental Drift to the Earth Sciences*, 37 (Academic Press, London, 1973).
- ²² Harland, W. B., in *Problems in Palaeoclimatology*, 119 (Interscience, London, 1964).
- ²³ Harland, W. B., *Geol. Rdsch.*, **54**, 45 (1965).
- ²⁴ Kroner, A., *Geol. Rdsch.*, **60**, 1513 (1971).
- ²⁵ Cahen, L., in *African Magmatism and Tectonics*, 97 (Oliver and Boyd, Edinburgh, 1970).
- ²⁶ Biju-Duval, B., and Gariel, O., *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, **6**, 283 (1969).
- ²⁷ Trompette, R., *C.r. hebdom. Séanc. Sci., Paris*, **275**, 1207 (1972).
- ²⁸ King, L. C., in *Descriptive palaeoclimatology*, 307 (Interscience, New York, 1961).
- ²⁹ Beuf, S., Biju-Duval, B., Stevaux, J., and Kulbicki, G., *Revue Inst. fr. Pétrole*, **21**, 363 (1966).
- ³⁰ Briden, J. C., *J. geophys. Res.*, **73**, 725 (1968).
- ³¹ Dunn, P. R., Thompson, B. P., and Rankama, K., *Nature*, **231**, 498 (1971).
- ³² Crawford, A. R., and Daily, B., *Nature*, **230**, 111 (1971).
- ³³ Robertson, W. A., and Fahrig, W. F., *Can. J. earth Sci.*, **8**, 1355 (1971).
- ³⁴ Irving, E., and Park, J. K., *Can. J. earth Sci.*, **9**, 1318 (1972).
- ³⁵ Bucha, V., *J. Geomagn. Geoelect. Kyoto*, **17**, 435 (1965).
- ³⁶ Al-Khafaji, S. A., and Vincenz, S. A., *Geophys. J. R. astr. Soc.*, **24**, 175 (1971).
- ³⁷ Howell, L. G., and Martinez, J. D., *Geophysics*, **22**, 384 (1957).

Chimaera mouse study shows absence of disease in genetically dystrophic muscle

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Chimaeras have been derived from aggregation of normal and dystrophic mouse embryos which had healthy muscles of almost totally dystrophic genotype, and diseased muscles of normal genotype. These results suggest that extramuscular factors must be implicated in muscular dystrophy.

UNTIL recently muscular dystrophy in man and animals was considered to result from a genetically determined primary defect within the muscle fibres themselves. Evidence is now accumulating which suggests that dystrophic individuals have an abnormality within the nervous system which may itself be largely responsible for the muscular disorder; namely, an abnormal trophic influence of the motor neurones on the affected muscles^{1,2}.

One ideal situation which could allow the investigation of the site of the primary genetic lesion would be an adult animal in which either genetically dystrophic muscle was innervated by a normal nervous system or genetically normal muscle was innervated by a dystrophic nervous system. The mouse chimaera offers an opportunity to approach this ideal situation without requiring that the animal undergo surgical manipulations, with subsequent establishment of regenerative processes³.

Mouse chimaeras are derived from the aggregation of pre-implantation embryos (each of eight cells) of differing

genotypes. These mosaic morula are cultured for 24 h (in which time they differentiate to single blastocysts) and are then transferred to the uterus of pseudopregnant females to complete development⁴. The chimaeras of interest here would derive from aggregation of dystrophic and normal embryo types. It was hoped that the resulting *in vivo* situation would make possible the study of the interaction between genetically defined muscle and the rest of the organism during development, and in the mature state.

In these chimaeras, if muscles of identified dystrophic genotype were found to have normal characteristics it would be unlikely that the disease could be due to a primary myopathy. In addition, if genetically normal muscle showed dystrophic characteristics, then this too would support the inference that there was an extramuscular component of the disease. Conversely, a direct correlation between dystrophic genotype and dystrophic characteristics would support the original myogenic hypothesis.

The principal requirement was to have an adequate genetic marker with which to determine the muscle genotype. Amongst the inbred strains of mice there are several suitable markers, and for this experiment the electrophoretic isozymes of malic enzyme⁵ were incorporated as the genotype marker, for the mature muscles of the dystrophic-normal chimaeras.

An important feature of the proposed *in vivo* preparation is that the muscle would be investigated at a time when, in the lifespan of an animal with the dystrophic genotype, the

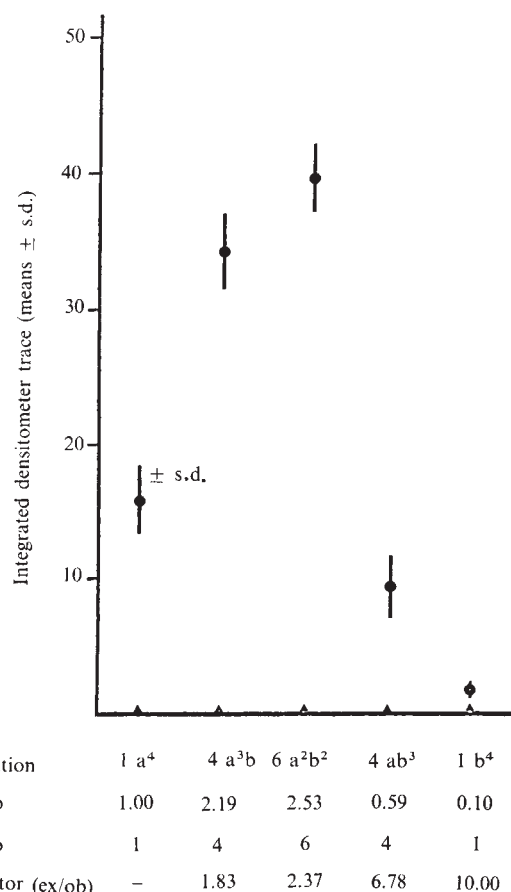


FIG. 1 Activity ratio of *Mod-I* isozymes in the heterozygote pattern from muscle supernatant. Correction factors were applied to densitometer results of chimera muscle before calculation of relative *Mod-I^a/Mod-I^b* nuclei.

disease would be clearly shown⁶. The slow clinical progression of the disease in dystrophic mice with the new *dy^{2J}* allele allows these mice to mate⁶, and homozygous dystrophic morula were obtained by direct mating of dystrophic pairs. For the experiment seven chimaeras of dystrophic $\longleftrightarrow$ normal (C57Bl/6J *dy^{2J}/dy^{2J}* $\longleftrightarrow$ SWV) and two of normal $\longleftrightarrow$ normal (C57Bl $\longleftrightarrow$ SWV) composition, all of which demonstrated coat colour and haemoglobin mosaicism, were produced. These were examined daily (birth to 2 months) and weekly for the following 4 to 10 months in different animals, for clinical features of muscular dystrophy.

An estimate of gross muscle function in the mature chimaeras was obtained by recording the maximum isometric twitch of their anterior tibialis muscles in response to supra-maximal nerve stimulation.

The histological appearances of the muscles from chimaeras were compared with those of normal and of dystrophic mice. Individual identified muscles were dissected out and a 2 mm section, taken across the middle of the muscles, was processed for subsequent preparation of 1 μ m Epon sections; the remainder of the muscle was frozen in liquid nitrogen and stored at -70° C for subsequent genotype analysis.

Genetic marker

The dystrophic embryos used in this chimera preparation had the *Mod-I^b* genotype for malic enzyme (E. S. Russell, personal communication). The normal embryos were determined to have a *Mod-I^a* genotype for the same enzyme. The subsequent analysis depended on the recognition and relative quantitation of the *Mod-I^a* and *Mod-I^b* gene products in the muscles investigated. This was achieved by starch gel electro-

phoresis, which enables the clear resolution of the isozymes of malic enzyme. *Mod-I^a* and *Mod-I^b* genes code for the subunits of the enzymatically active tetramere, malic enzyme⁷. In the *Mod-I^a/Mod-I^b* heterozygote where a and b subunits are simultaneously produced⁸ and randomly associate, five tetrameric forms, aaaa, aaab aabb, abbb, bbbb are electrophoretically resolved. In the dystrophic $\longleftrightarrow$ normal chimaeras, the presence of b subunits observed by resolution of the *Mod-I^b* isozyme or hybrid bands indicates the contribution of genetically dystrophic nuclei. Subunits of a type, forming the *Mod-I^a* isozyme or hybrid bands, could only be derived from the genetically normal nucleus. The descriptions of nuclei as 'normal' or 'dystrophic' is based upon this isozyme characterisation. The hybrid band feature of this isozyme system was used to differentiate muscles with mosaic fibres, that is, fibres with both normal and dystrophic nuclei within the same cytoplasm, from muscles where there were mixtures of fibres, each of which had only normal or dystrophic nuclei, but not both. In the latter case, only the parental bands, aaaa and/or bbbb would be detected⁹.

If the enzymatic activities of the five tetrameric types were equal, in the heterozygote, the theoretically expected 1:4:6:4:1 ratio from random association of the subunit types into tetrameres would be observed in the ratio of activity developed on the starch gel. This ratio was not observed in the original description of the *Mod-I* tetrameric types⁷ nor was it seen in the electrophoretic system used in the present experiment. The measured density ratio of the five bands in the heterozygote after enzymatic staining on the starch gel was 1.00 aaaa, 2.19 aaab, 2.53 aabb, 0.59 abbb, and 0.10 bbbb (Fig. 1). The assumption was made therefore that each tetramere type had a unique enzymatic activity. To measure the *Mod-I^a* and *Mod-I^b* gene products the observed activity for each tetrameric type was corrected by unique factors to regenerate the theoretical 1:4:6:4:1 ratio. These factors, derived from heterozygote electrophoretograms, were subsequently applied to the electrophoretograms of the chimera muscles before calculation of the relative *Mod-I^a* and *Mod-I^b* genotypes.

Muscle analysis

The behaviour of all the chimaeras was not distinguishable from that of normal animals of the same age. At no time could convincing features of muscular dystrophy be demonstrated by any of the recognised stresses and manipulations known⁶.

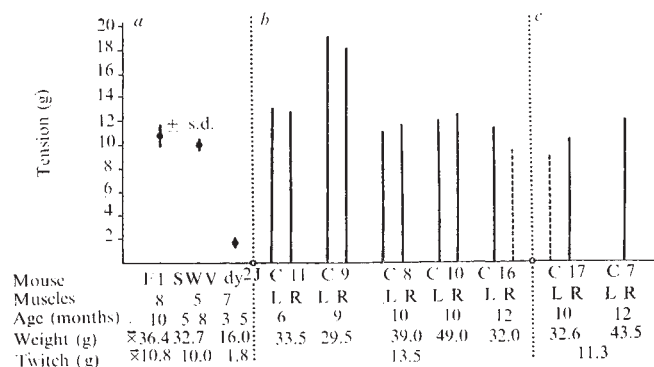


FIG. 2 Maximum isometric twitch of anterior tibialis muscles. Mouse: F1 is SWV-C57Bl/10J; *dy^{2J}* is C57Bl/6J *dy^{2J}/dy^{2J}* dystrophic; C is chimera (C11, 9, 8, 10, 16 are dystrophic $\longleftrightarrow$ normal; C17 and 7 are normal $\longleftrightarrow$ normal). Muscles: numbers are number of muscles analysed; L and R is left and right; broken lines represent suboptimal preparations and are not included in means; C7 left was a technical failure. a, control; b, dystrophic chimaeras; c, normal chimaeras.

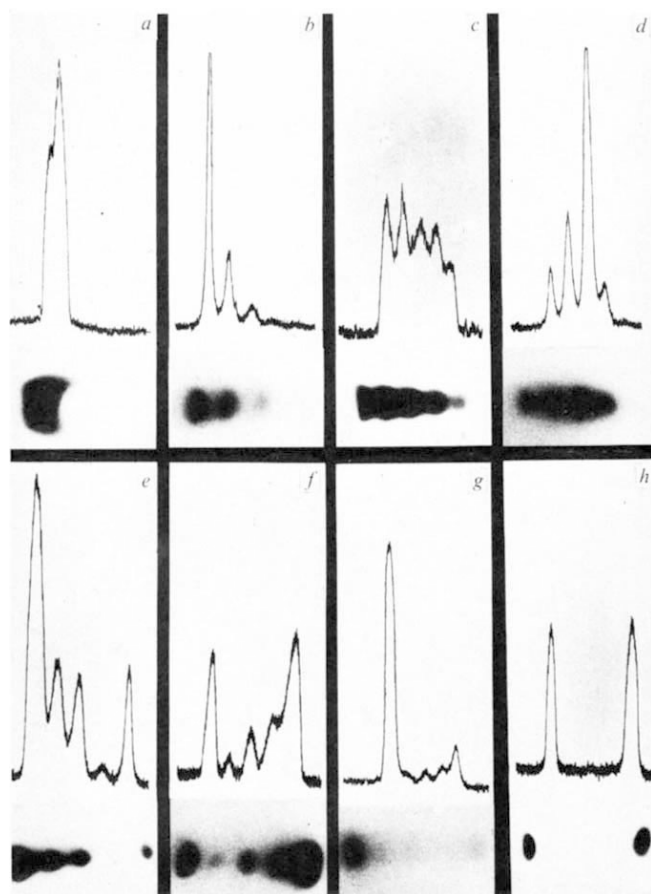


FIG. 3 *Mod-I* electrophoretograms and corresponding densitometer traces from C57Bl/6J $dy^{23}/dy^{23} \longleftrightarrow$ SWV $^{+/+}$ chimaera muscles. *Mod-I^a* isozyme is on the left of each trace. Frozen muscle samples were weighed, homogenised in 1 ml of H_2O ; centrifuged at $3^\circ C$ for 15 min at 15,000g; the supernatant at 90,000g for 30 min, and freeze dried. The supernatant was reconstituted with H_2O to yield a seven-fold concentration before electrophoresis. Electrophoresis was carried out on a microstarch gel system¹². The buffer system was modified for high voltage ($21 V cm^{-1}$ after Baker and Mintz⁹). The bridge consisted of 2,000 ml H_2O , 18.8 g citric acid (anhydrous) and Trisma base to pH 8.4. Gel buffer was 12.5 ml of bridge diluted to 250 ml with H_2O and re-adjusted to pH 8.4 with Trisma base. Electrophoresis was performed at $5^\circ C$ for 5 h. Malic enzyme was demonstrated by the assay of Henderson¹³. Densitometry was carried out directly with glycerine-cleared gels on a Joyce Loebel recording microdensitometer. The trace was integrated by planimetry and the relative a and b subunit composition was calculated.

All the anterior tibialis muscles investigated showed twitch tensions which were either equal to or even larger than those of normal animals which were examined in parallel (Fig. 2).

Electrophoretograms from the majority of dystrophic $\longleftrightarrow$ normal chimaera muscles revealed the activity of both dystrophic and normal nuclei (Fig. 3). The constitutional genotype of the muscles examined ranged from completely normal (Fig. 3a) through many heterozygous-like forms (Fig. 3b-g) to muscles with a mixed population of totally normal and totally dystrophic fibres (Fig. 3h). Although no muscles without some normal nuclei were observed, in some muscles as many as 94% of the nuclei were estimated to be of dystrophic genotype. Amongst these muscles with a high proportion of dystrophic nuclei, some had very large populations of fibres with only dystrophic nuclei (Fig. 3h). The dystrophic nuclei composition of 47 muscles, pooled from five chimaeras, was 58%. This estimate of dystrophic geno-

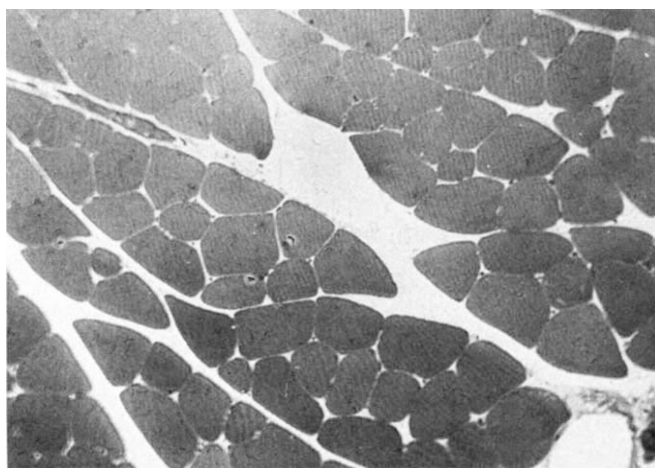


FIG. 4 Left biceps femoris muscle of chimaera No. 10. Note the normal morphology of muscle fibres. Magnification $\times 153$ approximately.

type indicates that no obvious selective death of genetically dystrophic nuclei had taken place either during myogenesis or in the mature muscle.

In the two normal $\longleftrightarrow$ normal chimaeras, in which the *Mod-I^b* subunits were derived from C57Bl nuclei without the dystrophic genotype, 10 muscles were pooled in a similar fashion for genotype analysis. In these, the *Mod-I^b* genotype was estimated to be 82%. This higher value for *Mod-I^b* nuclei probably reflects the small sample size, and not a selective advantage of *Mod-I^b* cells, since individual muscles of these normal chimaeras had from 3% to 94% *Mod-I^b* nuclei.

Since the hind limbs show the earliest and most extensive dystrophic clinical symptoms in dystrophic mice, histological examination was made of hind limb muscles in chimaeras which demonstrated extremes of genetic constitution. Minor features of dystrophic muscle pathology, such as central nucleation, were observed in very few fibres in every dystrophic $\longleftrightarrow$ normal chimaera muscle examined. No correlation of these minimal pathological features and the constitutional genotype was observed. Thus in Fig. 4, the left biceps femoris muscle of chimaera No. 10, had 83% dystrophic genotype, and the only detectable abnormality was the occasional central nucleus. Conversely, the left biceps femoris of chimaera No. 16, with no detectable dystrophic

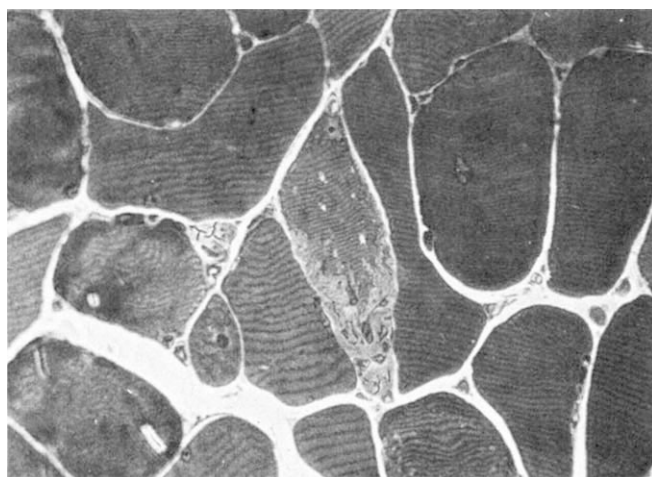


FIG. 5 Left biceps femoris muscle of chimaera No. 16. Note frequency of central nucleation and the presence of coagulation necrosis. Magnification $\times 510$ approximately.

genotype, demonstrated relatively more extensive pathological involvement than all the other muscles examined, that is, degenerating fibres and fibres with central nucleation grouped across the sections (Fig. 5).

These results from chimaeric mice are not compatible with a primary myopathy in murine muscular dystrophy. It could be argued that a small population of normal nuclei, which support synthesis of some postulated chemical, deficient within the fibres of dystrophic muscle, could prevent dystrophic pathology. This seems unlikely, however, because muscles with a high or very high dystrophic genotype, including those with large populations of completely dystrophic fibres, appeared otherwise normal. Furthermore, genetically normal muscle showed pathological changes; which suggests that, at least in the mosaic environment, some entirely extramuscular factor is primarily responsible for the muscle degeneration.

Finally, it has been suggested that these artificial chimaeras show mosaicism similar to that expressed in human females as a consequence of random inactivation of one X chromosome¹⁰. A variable muscle constitutional genotype has long been considered to account for the variable pathological features observed in females carrying the X-linked form of Duchenne muscular dystrophy¹¹. If the primary lesion in Duchenne dystrophy is homologous to that in murine dystrophy, this concept must be re-evaluated. A variable mosaicism in some other cell type, possibly the motor neurones, could account for this observed clinical variability.

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- ¹ McComas, A. J., Sica, R. E. P., and Campbell, M. H., *Lancet* **i**, 321-325 (1971).
- ² Gallup, B., and Dubowitz, B., *Nature*, **243**, 287-289 (1973).
- ³ Salafsky, B., *Nature*, **299**, 270-272 (1971).
- ⁴ Tarkowski, A. K., *Nature*, **190**, 857-860 (1961).
- ⁵ Shows, T. B., Chapman, V. M., and Ruddle, F. H., *Biochem. Genet.*, **4**, 707-718 (1970).
- ⁶ Meier, H., and Southard, J. L., *Life Sci.*, **9**, 137-144 (1970).
- ⁷ Shows, T. B., and Ruddle, F. H., *Science*, **160**, 1356-1357 (1968).
- ⁸ Engle, W., and Wolfe, U., *Humangenetik*, **12**, 162-166 (1971).
- ⁹ Baker, W. B., and Mintz, B., *Biochem. Genet.*, **2**, 351-360 (1969).
- ¹⁰ Gearhart, J. D., and Mintz, B., *Dev. Biol.*, **29**, 27-37 (1972).
- ¹¹ Emery, A. E. H., *Lancet*, **i**, 884 (1964).
- ¹² Tsuyuki, H., Roberts, E., Kerr, R. H., and Ronald, A. P., *J. Fish. Res. Bd Can.*, **23**, 929-933 (1966).
- ¹³ Henderson, N. S., *Archs Biochem. Biophys.*, **117**, 28-33 (1966).

Gene dosage compensation in metafemales (3X; 2A) of *Drosophila*

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The X chromosome of Drosophila provides a unique system for the study of modulation of eukaryotic gene activity.

IN *Drosophila*, dosage compensation, that is, the equalisation of X-linked gene products in males and females, has been reported for RNA synthesis, enzyme activities and terminal phenotypic products¹⁻³. In contrast to mammals, where dosage compensation seems to be mediated by inactivation of one of the two X chromosomes in somatic cells of diploid females, there is good evidence that in *Drosophila* both X chromosomes are active in all cells of the female soma⁴ although the level of activity of each is half that of the single X in a male.

This observation has been extended recently to triploid females (3X;3A where A is one set of autosomes)⁵⁻⁸. Furthermore, in male-like triploid intersexes (2X;3A), the X chromosome was reported to function at a level intermediate between the normal male and female levels. Thus X-linked gene activity seems to be correlated with the rela-

tive number of X chromosomes and sets of autosomes in the genome: activity is high in males where the X/A ratio is 0.5, intermediate in triploid intersexes where the X/A ratio is 0.66 and low in diploid or triploid females where the X/A ratio is 1.0. To test this correlation we have measured X-linked and autosomal gene activity in metafemales (3X;2A) where the higher X/A ratio of 1.5 might be expected to yield a relative activity per gene dose even lower than that characteristic of females. That this may be the case was suggested by the observation that metafemales and females homozygous for an eye colour hypomorphic mutation have the same phenotype⁹. Our results indicate that enzyme activity and rates of RNA synthesis are similar in metafemales and in normal females and, therefore, that X-linked gene activity expressed on a per gene basis is less in these metafemales than in diploid or triploid females.

We used laboratory stocks of *D. melanogaster* and the genetic markers and symbols are those described by Lindsley and Grell¹⁰. Various doses of the structural gene for 6-phosphogluconate dehydrogenase¹¹ (*Pgd*⁺) were obtained using *T*(1 → 3)*w*^{sc}. This rearrangement consists of a transposition of a small segment of the X chromosome, including *Pgd*⁺, into chromosome 3. The two elements of the

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TABLE 1 6PGD activity for various structural gene doses in male and female larvae

Sex	Experiment†	No. of <i>Pgd</i> ⁺	6PGD	Enzyme activities*		IDH
				G6PD‡	ADH	
Males	A	1	6.3 ± 0.2	7.1 ± 0.1	35.9 ± 0.5	14.6 ± 0.3
		2	13.0 ± 0.6	8.4 ± 0.1	38.8 ± 0.9	17.8 ± 0.3
	B	1	6.0 ± 0.4	7.4 ± 0.3	31.0 ± 1.8	14.9 ± 0.7
		2	11.5 ± 0.5	8.3 ± 0.0	35.3 ± 1.1	17.7 ± 0.8
Females	A	2	6.4 ± 0.4	6.9 ± 0.2	35.6 ± 1.0	16.2 ± 0.4
		1	3.5 ± 0.2	6.2 ± 0.3	33.8 ± 2.5	17.4 ± 0.5
	B	2	5.9 ± 0.5	6.4 ± 0.2	38.1 ± 4.3	13.6 ± 0.8
		3	9.2 ± 0.3	7.6 ± 0.1	39.3 ± 1.6	17.2 ± 0.4

* Expressed as mean μmol coenzyme reduced $\times 10^2$ per min per mg protein $\pm$ s.e. (No. of determinations per mean = 4.)

† Experiment A, flies produced by mating *e/e* females to *Df(1)w^{co}, v f/Y; Dp(1 → 3)w^{co}, e⁺/e* males. Experiment B, flies produced by mating *e/e* females to *y w spl/Y; Dp(1 → 3)w^{co}, e⁺/e* males.

‡ These values are corrected for an estimated 6% level of 6PGD activity in the G6PD assay using an extract from normal females.

translocation are *Df(1)w^{co}*, the deficient X chromosome, and *Dp(1 → 3)w^{co}*, the duplication-bearing autosome. Females homozygous for the recessive marker *ebony* (*e*) were crossed to either *Df(1)w^{co}, v f/Y; Dp(1 → 3)w^{co}, e⁺/e* males or to *y w spl/Y; Dp(1 → 3)w^{co}, e⁺/e* males. Full-grown third instar larvae were collected and sorted according to sex and ebony (black spiracles) or non-ebony (wild type, brownish spiracles) phenotype. The first cross yields ebony male and female larvae bearing one dose of *Pgd*⁺ and non-ebony male and female larvae bearing two *Pgd*⁺ doses. The second cross yields ebony male larvae bearing one *Pgd*⁺ dose, non-ebony male and ebony female larvae bearing two *Pgd*⁺ doses, and non-ebony female larvae possessing three doses of *Pgd*⁺.

To produce metafemalae larvae, *C(1)RM, y v f/Y* attached-X females were crossed to *y v f·y/Y* males. (The X chromosome represents an X-ray-induced detachment of the attached-X, marked with a small duplication bearing the wild type, dominant allele of *yellow, y⁺*.) Female larvae with yellow mouth parts and setae were controls (2X:2A), those with black mouth parts and setae were metafemales, while all male larvae had black mouth parts. To improve the yield of emerged adult metafemales, *C(1)RM, y v f/Y* females were outcrossed to *+/Y* males of a Samarkand wild-type line. The phenotypic distinction of the various larval types was the same as in the previous cross. While crosses were usually incubated at $25^\circ \pm 1^\circ \text{C}$, some cultures of attached-X females by wild type males were maintained at $20^\circ \pm 1^\circ \text{C}$ to obtain larvae for autoradiography.

Larval samples were homogenised in conical ground-glass

homogenisers at a ratio of fifteen larvae per ml of distilled water; adult samples were homogenised at a concentration of 20 mg live weight per ml of distilled water. After 20 min, samples were centrifuged for 40 min at 12,000 *g* and the clear supernatant was used as a crude enzyme extract. Throughout preparation all extracts were maintained at 0° – 5°C .

Larval fat body samples were obtained by chopping 50 mature third instar larvae with a razor blade in a few drops of 2% sucrose. After transfer to a test tube containing approximately 5 ml of cold 2% sucrose, fat bodies and other organ fragments were dislodged from the body wall casings by aspirations into a Pasteur pipette. After standing for 20 min at 0°C , most of the loose fat body fragments floated to the surface, remarkably free of other tissue. The fat body layer was drawn off and its volume adjusted to 1 ml with distilled water. After brief sonication, the extract was centrifuged for 20 min at 12,000 *g* and enzyme activities were determined in the supernatant.

We measured activities of glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), α -glycerophosphate dehydrogenase (α -GPDH, EC 1.1.1.8), NADP-dependent isocitrate dehydrogenase (IDH, EC 1.1.1.42), and alcohol dehydrogenase (ADH, EC 1.1.1.1). Substrate solutions and assay conditions for the first four enzymes have been described before⁶. ADH was measured using the reaction mixture of Jacobson *et al.*¹². All dehydrogenase activities were monitored spectrophotometrically at 340 nm and enzyme activities are expressed as μmol of NAD⁺ or NADP⁺ reduced

TABLE 2 Enzyme activities in metafemales and euploid controls

Material	Sex	6PGD	Enzyme activities*		IDH
			G6PD	α -GPDH	
Whole larvae	Males	6.6 ± 0.2† (5)	8.0 ± 0.6 (7)	16.3 ± 1.1† (7)	21.3 ± 0.3 (7)
	Females	7.3 ± 0.2† (5)	7.4 ± 0.4 (10)	16.2 ± 0.8† (10)	22.3 ± 0.6 (10)
	Metafemales	8.5 ± 0.3 (4)	8.0 ± 0.3 (9)	22.3 ± 1.1 (9)	23.8 ± 0.6 (9)
Fat body	Males	9.4 ± 0.4† (4)	7.0 ± 0.3† (4)	14.3 ± 1.0† (4)	11.9 ± 0.6† (4)
	Females	9.1 ± 0.7† (4)	7.6 ± 0.5† (4)	14.6 ± 0.8† (4)	11.6 ± 0.4† (4)
	Metafemales	14.4 ± 0.5 (5)	10.8 ± 0.2 (5)	23.4 ± 0.7 (5)	13.9 ± 0.4 (5)
0–1 h adults	Males	7.4 ± 0.2 (6)	6.4 ± 0.2 (6)	63.9 ± 2.4† (6)	29.4 ± 0.7† (6)
	Females	7.6 ± 0.2 (6)	8.4 ± 0.2 (6)	59.9 ± 2.0† (6)	29.3 ± 0.3† (6)
	Metafemales	7.4 ± 0.4 (5)	7.0 ± 0.7 (5)	44.6 ± 1.2 (5)	24.4 ± 0.8 (5)
9–11 h adults	Males	10.1 ± 0.6 (2)	8.3 ± 0.4 (2)	97.7 ± 3.6 (2)	35.8 ± 1.0 (2)
	Females	8.6 ± 0.5 (2)	9.4 ± 0.4 (2)	86.4 ± 3.2 (2)	33.4 ± 1.6 (2)
	Metafemales	9.2 ± 0.1 (2)	10.1 ± 0.1 (2)	77.2 ± 1.2 (2)	34.6 ± 0.0 (2)

* Expressed as mean μmol coenzyme reduced $\times 10^2$ per min per mg protein $\pm$ s.e. Number of determinations are indicated in parentheses.

† Male or female values differ from metafemale values at the 0.05 level of significance.

TABLE 3 Normalised X-linked enzyme activities per gene dose in metafemales and females

Material	Sex	6PGD/ α -GPDH	6PGD/IDH	G6PD/ α -GPDH	G6PD/IDH
Whole larvae	Females	0.21 $\pm$ 0.03	0.17 $\pm$ 0.02	0.23 $\pm$ 0.02	0.17 $\pm$ 0.02
	Metafemales	0.11 $\pm$ 0.01	0.11 $\pm$ 0.01	0.12 $\pm$ 0.01	0.11 $\pm$ 0.01
Fat body	Females	0.31 $\pm$ 0.04	0.39 $\pm$ 0.03	0.26 $\pm$ 0.02	0.34 $\pm$ 0.05
	Metafemales	0.21 $\pm$ 0.01	0.37 $\pm$ 0.03	0.16 $\pm$ 0.02	0.26 $\pm$ 0.03
0-1 h adults	Females	0.063 $\pm$ 0.003	0.13 $\pm$ 0.01	0.071 $\pm$ 0.003	0.14 $\pm$ 0.01
	Metafemales	0.055 $\pm$ 0.010	0.10 $\pm$ 0.01	0.052 $\pm$ 0.014	0.09 $\pm$ 0.02
9-11 h adults	Females	0.051 $\pm$ 0.002	0.13 $\pm$ 0.01	0.054 $\pm$ 0.000	0.14 $\pm$ 0.01
	Metafemales	0.040 $\pm$ 0.004	0.09 $\pm$ 0.003	0.044 $\pm$ 0.002	0.10 $\pm$ 0.00

Activities are expressed as mean standardised units $\pm$ s.e.

per min per mg protein at 29° C. Extract protein concentration was determined using the method of Lowry *et al.*¹³. Mean enzyme activity values were compared using Student's *t* test.

For autoradiography, salivary glands were dissected out of late third instar larvae in Ephrussi-Beadle solution. They were incubated for 5 min in ³H-uridine (Amersham Searle, 30 Ci mmol⁻¹) at a concentration of 20 μ Ci ml⁻¹. Fixation and squashing, preparation, exposure and development of autoradiographs were as previously described⁷. The rate of RNA synthesis was measured in segment 1-3C of the X chromosome using an autosomal segment—the right arm of chromosome 2 (58F-60)—as an internal control. Grains were counted in nuclei where both segments were unobstructed. Average values of grain counts over the X segment ($\bar{X}$) and the autosomal segment ($\bar{2R}$) were obtained for every gland. The slope of the regression line of $\bar{X}$ on $\bar{2R}$ is a measure of the average increment in the number of grains over the X which accompanies a unitary increment in the number of grains over the autosome. This slope, represented by the regression coefficient *b*, is a measure of the rate of RNA synthesis in the X relative to that in an autosome.

Enzyme levels

Before examining the dosage compensation of metafemale enzyme levels in larvae, we had to establish that the relationships between regular adult male and female enzyme levels are also evident in larvae. As Table 1 shows, we found this to be the case. Two of the four enzymes studied, 6PGD and G6PD, are X-linked^{11,14} while the known structural genes for ADH and IDH are linked to chromosomes two and three, respectively^{15,16}. The autosomal enzyme levels of male and female larvae are equivalent and dosage compensation seems to be fully operative, as evidenced by the similarity in G6PD and 6PGD activities in the two sexes, and by the observation that each *Pgd*⁺ dose in female larvae with one, two or three doses of this gene produces approximately half the amount of 6PGD that it produces in male larvae with one or two doses of the gene.

Enzyme activities of metafemales and normal diploid females are presented in Table 2. Activities of male sibs are

included as an internal control. Measurements were performed on extracts of whole larvae, larval fat body, 0-1 h and 9-10-h-old emerged adults. α -GPDH, the gene for which is located on the second chromosome¹⁷, was measured instead of ADH in these experiments. No male and female values differed from each other. Metafemales, on the other hand, showed greater variability, with values often in excess of those of control females. In most cases, however, both X-linked and autosomal enzymes seemed to be affected. For example, metafemale larvae have significantly higher specific activities of 6PGD and α -GPDH than euploid controls. This may be the consequence of the longer time required by metafemales to reach the end of the third larval instar, leading to the preferential accumulation of certain soluble proteins. When we measured enzyme activities in the larval fat body, which is metabolically highly active, all soluble dehydrogenases assayed were significantly higher in metafemales than in euploid controls. Emerged adult metafemales, on the other hand, had activities either reduced (α -GPDH and IDH in 0-1 h-old adults) or equivalent to those of controls.

X-linked enzyme activity measurements can be normalised in relation to either α -GPDH or IDH activity, since metafemales and control females each contain two doses of the structural genes of these enzymes per genome. Table 3 contains per gene activities obtained by dividing the normalised X-linked activities of metafemales by three and of females by two. Taken together and evaluated in the light of the generalised physiological disturbances of metafemales, these results indicate that X-linked genes are substantially less active in metafemales than in normal females, and that the presence of a third X chromosome in the genome of the former flies does not mediate a structural gene dosage response, that is, a 50% increase in X-linked gene product per cell. This conclusion is substantiated by the autoradiographic monitoring of X chromosome activity.

RNA synthesis

The results of the study of ³H-uridine incorporation by metafemale and diploid female larval salivary gland chromosomes are presented in Table 4. To compare metafemale and female grain counts properly, it must be remembered that in the former the X element contains three synapsed chromo-

TABLE 4 RNA synthesis in metafemales and females

Sex	No. nuclei	No. glands	Average [X]	Average [2R]	<i>r</i>	<i>b</i> $\pm$ s.e.
Metafemales	76	9	59.17	44.74	0.98	0.96 $\pm$ 0.07
Females	84	9	60.52	52.13	0.97	1.10 $\pm$ 0.10

Average [X] and average [2R] = overall average of grain counts over X and 2R segments, respectively.

r, Correlation coefficient of the mean grain counts for individual glands, [X] and [2R].

b, Regression coefficient of [X] on [2R]. Not significantly different.

The metafemale values for average [X] and *b* were corrected for differential self-absorption.

somes instead of two and therefore absorbs more β particles than does the X element of control females. Taking the efficiency of X chromosome grain production in males as approximately 20% higher than in females⁷, the values for meta-females were corrected by an increment of 10%.

If the grain counts over the X and autosome are averaged for each gland and a regression line of these averages ($\bar{X}$ on $[2R]$) is established, the slope of this line is the same in males and females (G. Maroni, R. Kaplan and W. Plaut, in preparation) or in triploid intersexes and triploid females⁷. This indicates that the rate of RNA synthesis by the X element relative to the rate of synthesis by the autosomal element is the same in males where the X is single as in females where the X element is made up of two paired X chromosomes; similar conclusions were reached for triploid intersexes (two Xs) and triploid females (three Xs). We

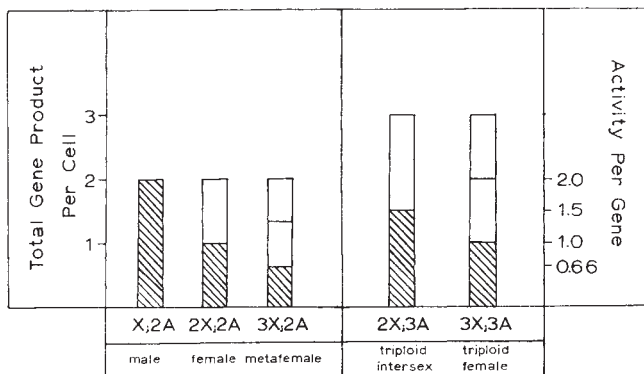


FIG. 1 Schematic representation of X gene activity in individuals of various chromosomal constitutions. The height of the columns represents total gene product per cell; a value of 2 was arbitrarily chosen for diploids. The shaded areas represent activity per gene dose; diploid females were given a value of 1.

found that there was no significant difference between the regression coefficients of metafemales and females, indicating that the rate of RNA synthesis by the trivalent X element of metafemales relative to the rate of RNA synthesis by an autosomal element is indistinguishable from that of normal females. Another way of expressing this result would be to say that an X chromosome in a metafemale synthesises, per unit time, only two-thirds of the RNA which it normally does in a female.

Compensation and modulation

When our findings are pooled with those derived from enzymological and autoradiographic comparisons of diploid males and females^{1,2}, it is evident that the overall expression of X-linked genetic activity (that is, the total amount of X-linked gene product) in diploid cells is independent of the number of X chromosomes within the cell. Similarly, comparisons of triploid genotypes⁶⁻⁸ suggest that within triploid cells, the phenotypic level achieved by X-linked genes is independent of the number of X chromosomes present in the cell. In triploid cells, however, the overall level of X-linked gene activity is approximately 50% greater than that of diploid cells^{5,8}. Because of the latter relationship, Maroni and Plaut^{7,8} proposed that dosage compensation is mediated by an autosomally-derived factor which is rate-limiting in the expression of X-linked genes. Our current finding with respect to the equivalence of X-linked activity

in metafemales and diploid females agrees with this hypothesis.

In spite of differences in the means of implementation of dosage compensation, there is a similarity between *Drosophila* and mammals. Lyon¹⁸ has suggested that the somatic differentiation of X chromosomes into active or inactive states involves interplay of genes on the X and an autosome. This suggestion is based on the observation that in human aneuploids with two sets of autosomes and varying numbers of sex chromosomes (XXY; 2A,3X; 2A or 4X;2A) only one X remains active, while in triploids (XXY;3A or 3X;3A) or tetraploids (XXYY;4A) two X chromosomes can remain active^{19,20}.

The modulation of gene activity is a central problem in the study of genomic expression. Different levels of induction can be achieved easily with microbial systems by manipulation of effector or terminal product concentrations. Analogous circumstances are much more difficult to establish in the case of multicellular organisms. Therefore, the X chromosome of *Drosophila* is of unique interest in this respect since four levels of activity have been recorded for genes on this chromosome: the highest level is in males, the lowest in metafemales, and two intermediate levels are in triploid intersexes and euploid females (Fig. 1). These relative levels of activity seem to be established comparatively early in development, before the late larval stages. Elucidation of the causative factors responsible for a given level of X-linked gene activity in *Drosophila* should contribute to the understanding of other examples of eukaryotic genome modulation.

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- Mukherjee, A. S., and Beermann, W., *Nature*, **207**, 785 (1965).
- Seecof, R. L., Kaplan, W. D., and Futch, D. G., *Proc. natn. Acad. Sci. U.S.A.*, **62**, 528 (1969).
- Muller, H. J., *Harvey Lectures*, Series XLIII, 165 (1950).
- Kazazian, H. H., Young, W. J., and Childs, B., *Science*, **150**, 1601 (1965).
- Lucchesi, J. C., and Rawls, jun., J. M., *Biochem. Genet.*, **9**, 31 (1973).
- Lucchesi, J. C., and Rawls, jun., J. M., *Genetics*, **73**, 459 (1973).
- Maroni, G., and Plaut, W., *Chromosoma*, **40**, 361 (1973).
- Maroni, G., and Plaut, W., *Genetics*, **74**, 331 (1973).
- Stern, C., *Can. J. gen. Cytol.*, **2**, 105 (1960).
- Lindsley, D. L., and Grell, E. H., *Carnegie Institute of Washington Publication*, No. 627 (1968).
- Young, W. J., *J. Hered.*, **57**, 58 (1966).
- Jacobson, K. B., Murphy, J. B., and Hartman, F. C., *J. biol. Chem.*, **245**, 1075 (1970).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).
- Young, W. J., Porter, J. E., and Childs, B., *Science*, **143**, 140 (1964).
- Grell, E. H., Jacobson, K. B., and Murphy, J. B., *Science*, **149**, 80 (1965).
- Fox, D. J., *Biochem. Genet.*, **5**, 69 (1971).
- Grell, E. H., *Science*, **158**, 1319 (1967).
- Lyon, M. F., *Biol. Rev.*, **47**, 1 (1972).
- Bomsl-Helmreich, O., *Adv. Biosci.*, **6**, 381 (1971).
- Carr, D. H., *J. med. Genet.*, **8**, 164 (1971).

LETTERS TO NATURE

PHYSICAL SCIENCES

Statistical analysis of close pairs of QSOs

THE observation of close pairs of QSOs with very different redshifts has been suggested to be evidence in support of the noncosmological redshift hypothesis^{1,2}. The statistical arguments used by Stockton¹ and by Wampler *et al.*² have recently been attacked by Bahcall and Woltjer³ on the ground that the method used is one of *a posteriori* statistics. Statistics aside, they have ignored the point that in one case², the evidence is not only based on close proximity of the two QSOs but also on two coincidences in wavelengths in the spectra of the two objects. Although the argument against *a posteriori* statistics is correct in the absolute sense, this method is widely used in astronomy and has generally been accepted, except in cases in which the hypothesis under consideration has not reached the point of general acceptability. The redshift controversy is a good example of this. Evidence as to the existence of non-cosmological redshifts must, because we have few ways of measuring distance, depend upon finding conflicts between distances derived from the redshifts (assumed cosmological) and distances derived in other ways⁴—either through statistical arguments or by finding luminous connections between pairs of objects. If, as seems to be the case, most workers use the redshift as the prime criterion of distance, and subordinate other methods of measurement to it, no solution to the problem is possible.

One or two examples of the prejudicial approach used in this problem will suffice:

(1) Several cases of QSOs with low redshift close to small groups of galaxies, and with redshifts similar to those of one or more members of the respective groups, have been found. Although some of these associations may be real, the statistical evidence is not convincing⁵; nevertheless, these juxtapositions are cited by many as proof of the cosmological origin of QSO redshifts. It is instructive to examine some of the analyses which attached statistical significance to such associations. For example, Gunn⁶ found a small group of galaxies near the QSO PKS2251+11 ($z = 0.33$) agreeing in redshift within the observational uncertainty ($\Delta z \approx \pm 0.01$). He finds that the brightest member of the group, with a calculated absolute magnitude -21.2 , lies $28''$ from the QSO; then he asks: "What is the probability of finding a galaxy of absolute magnitude -21 or brighter within $30''$ of a random position and within $3,000 \text{ km s}^{-1}$ of a given redshift near 0.3 ?" The answer, 0.001 before multiplying by the number of tries, is then purported to be "a realistic assessment of the probability of a chance coincidence". While the choice of $3,000 \text{ km s}^{-1}$ can be justified since it follows from the observational uncertainty, the choices of the magnitude limit, -21 , and the separation limit $30''$ are obviously predicated upon the observed values -21.2 and $28''$, respectively, and thus constitute *a posteriori* information.

(2) Another good example is found in a paper by Wolf and Bahcall⁷. They wish to argue that a galaxy in a cluster is physically associated with the N-system Ton256. We quote them as follows: "There appears to be a galaxy about $8''$ from Ton256; the redshifts of the galaxy and Ton256 are identical, to within the observational error of 300 km s^{-1} . The density of galaxies in the central region of the cluster is $\sim 0.08 \text{ galaxies (arc min)}^{-2}$; so the probability of having a galaxy

appear within $8''$ from the QSO, assuming a random distribution of the galaxies near the center of the cluster, is only about 0.4% . We thus assume that the galaxy is really associated with the QSO and is in a bound orbit around the QSO." Again, this is a classic piece of statistical analysis argued *a posteriori*, presumably considered acceptable because the redshifts of the two objects are the same.

In what follows we reconsider the pairs already discovered and then make an *a priori* calculation based on realistic parameters such that a test involving the discovery of further close pairs of QSOs may enable a significant statement to be made.

Let $\Gamma(< m)$ be the sky density of QSOs brighter than m . Then the expected number lying by chance within an angular separation θ of N arbitrary coordinates is

$$\langle n \rangle = 2.4 \times 10^{-7} N \Gamma(< m) \theta^2, \quad (1)$$

with Γ expressed in $(\text{arc deg})^{-2}$ and θ , in arc s. A plot of $\langle n \rangle$ against θ for various $N\Gamma$ is shown in Fig. 1. For N corresponding to the number of QSOs with known redshifts (~ 300), and $\Gamma(m_b < 19.4)$ taken from the Sandage-Luyten survey [$\sim 5 \text{ QSO (arc deg)}^{-2}$], the value for the product $N\Gamma$ is $\sim 1,500 \text{ tries per QSO (arc deg)}^{-2}$. This number could be increased to $\sim 3,000$ by extending the survey to $m_b < 20$. Of course, it is always possible to consider a more restricted sample, yielding a smaller value for $N\Gamma$, but to obtain a value much larger than $3,000$ would require deep plates

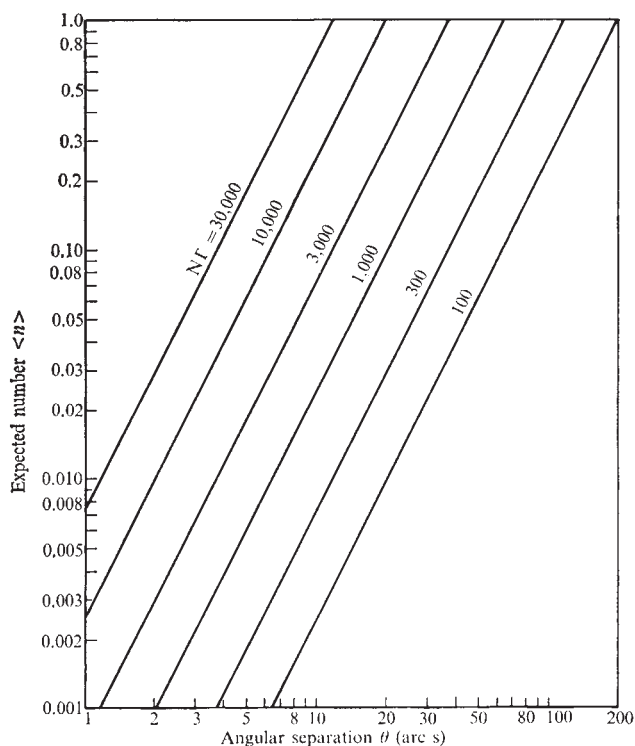


Fig. 1 Dependence of the expected number of apparent pairs upon pre-selected class intervals defined by the maximum angular separation θ to be included and the product of the number of tries N and the sky density $\Gamma(< m)$ of QSOs brighter than the adopted completeness limit m of the sample to be considered.

or deep scans around each of the known QSOs. From a practical point of view it would be difficult to go to $m_b = 21$ on the Palomar Sky Survey because objects at the very limit of the Survey, lying say $5''$ from a brighter object, will tend to be missed and in any case would be very difficult to examine spectroscopically.

The QSO 1548+115a lies about $5''$ from the previously identified QSO 1548+115b (ref. 2). Using the observed separation, the expected number of such pairs, for NT ($m > 19.4$) $\approx 1,500$, is only about ~ 0.01 ; however, since this choice of separation θ is based on *a posteriori* information, the quoted probability is only an approximate lower limit to a realistic *a priori* probability. In order to estimate an upper limit to the *a priori* probability, we can consider the class interval determined by the next nearest separation known—namely, the $35''$ separation of Ton155 and Ton156 (ref. 1). Adopting $\theta = 35''$, the number of pairs with smaller separations expected by chance is ~ 0.4 (see ref. 3).

The statistical significance of the pair 1548+115a,b is thus not well defined; it ranges from $\sim 99\%$ confidence to about 60% . Consequently, the *a priori* statistical significance of this pair cannot be demonstrated. Nevertheless, the proximity of the association is curious, particularly in view of the spectroscopic coincidences already mentioned. Unless additional evidence is uncovered, however, the proximity of 1548+115a,b will remain just a curiosity cited by some as evidence for non-cosmological redshifts, but disregarded by others as a mere coincidence. We therefore propose the statistical test described below.

The vicinity of all ~ 300 QSOs with known redshifts has not been examined closely, and, in fact the method of identification of QSOs from radio positions may have biased the sample against the discovery of close pairs because when a suggested identification close to the radio position is found to be a QSO, no further examination of other nearby objects is usually undertaken. Consequently, more associations may exist (compounding the probabilities). We propose, therefore, that the vicinity around each known QSO be examined for possible conjugates down to $m_b \approx 20$, since it should be feasible to examine any such candidates spectroscopically. For NT $\approx 3,000$, the expected number with chance separations less than $\theta = 10$ arc s is only $\langle n \rangle \approx 0.08$ (see equation 1 and Fig. 1). Thus, the discovery of a second pair of QSOs—or two more pairs of QSOs if the known case 1548+115a,b is excluded from the sample—would be statistically significant at 99.5% confidence, based on *a priori* class intervals.

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Absorption of high energy heavy nuclei and γ rays at the surface of hot neutron stars

SINCE the discovery of pulsars and their association with rotating magnetic neutron stars, it has been realised that pulsars can efficiently accelerate particles to high energies and effectively contribute to the observed cosmic-ray density in the Galaxy. Acceleration of particles to very high energies by the low frequency electromagnetic waves beyond the light cylinder is one mechanism proposed^{1,2}. Others³⁻⁵ have considered the possibility of accelerating particles by the electric field near the surface of the neutron star arising from non-zero $\mathbf{E} \cdot \mathbf{B}$. High energy photons are also likely to be produced in association with these particles and these have been observed from the Crab pulsar. Presumably, younger pulsars may radiate larger numbers of more energetic γ rays.

Though pulsars are potential sources of very high energy particles and γ rays, it is not clear whether the radiation can escape the pulsar environment and reach interstellar space. Various absorption processes can contribute to the destruction of these radiations. Absorption of high energy γ rays in their interactions with the radiation field of the pulsar may occur⁶⁻⁸. Destruction of high energy cosmic rays, especially the photodisintegration of heavy nuclei in the vicinity of pulsars, has also been considered^{9,10}. Heavy nuclei may be destroyed by fragmentation in the high density nebular matter surrounding pulsars in their early stages¹¹. All these processes become important in the early stages of pulsars because of the simultaneous presence of particles of very high energy and large radiation and matter density.

Here I point out that high energy heavy nuclei and γ rays may also be absorbed because of their interactions with the blackbody photons emitted from the surface of the hot neutron star. Neutron stars have surface temperatures greater than 10^7 K when they form and subsequently the temperature decreases¹². Tsuruta *et al.*¹³ have carried out improved cooling calculations taking into account the influence of superfluidity of the matter of the neutron star, the magnetic field and other factors. Their calculations show that for the first 100 yr the surface temperature is 10^7 K and remains greater than 5×10^6 K for another few hundred years for several models. So there is a high density of photons close to the neutron star during the early stages. It is precisely during this period that pulsars lose most of their energy and accelerate particles to high energies. I have discussed the implication of high surface temperature for the radio emission of pulsars elsewhere¹³; here I concentrate on other effects.

If a is the radius of the neutron star and T its surface temperature, the differential spectrum of photons emitted from the surface is

$$n(\epsilon) d\epsilon = \frac{2\epsilon^2}{h^3 c^2} (e^{\epsilon/kT} - 1)^{-1} \text{ photons (cm}^2 \text{ s sr erg)}^{-1} \quad (1)$$

Considering the interaction between a γ ray at O, at a distance R from the centre, travelling radially, and photons from the surface of the neutron star (see Fig. 1) the optical depth for γ - γ interaction is

$$\frac{d\tau}{dR} = \iint (2\pi a^2 \sin \alpha d\alpha \cos \phi) (r^{-2}) (1 - \cos \beta) n(\epsilon) d\epsilon \frac{\sigma(E_\gamma, \epsilon)}{c} \quad (2)$$

Here $\sigma(E_\gamma, \epsilon)$ is the cross section for pair production in the collision of a γ ray of energy E_γ with a photon of energy ϵ , the term $(2\pi a^2 \sin \alpha d\alpha \cos \phi)$ is the element of area normal to OA, the term r^{-2} represents the inverse square reduction and the term $(1 - \cos \beta)$ takes care of the relative velocity between the two photons. The integration with respect to α

¹ Stockton, A. N., *Nature phys. Sci.*, **238**, 37 (1972).

² Wampler, E. J., Baldwin, J. A., Burke, W. L., Robinson, I. B., and Hazard, C., *Nature*, **246**, 203 (1973).

³ Bahcall, J. N., and Woltjer, L., *Nature*, **247**, 22 (1974).

⁴ Burbidge, G. R., *Nature phys. Sci.*, **246**, 17 (1973).

⁵ Burbidge, G. R., and O'Dell, S. L., *Astrophys. J. Lett.*, **182**, L47 (1973).

⁶ Gunn, J. E., *Astrophys. J. Lett.*, **164**, L113 (1971).

⁷ Wolf, R. A., and Bahcall, J. N., *Astrophys. J.*, **176**, 577 (1972).

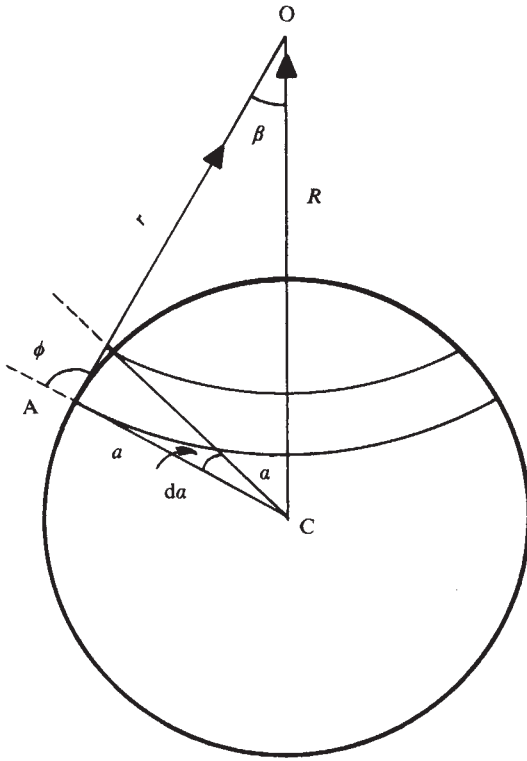


FIG. 1 The interaction between a γ ray or heavy nucleus with blackbody photons from an elemental surface of the neutron star (see text).

is to be carried out between the limits $\alpha = 0$ and $\alpha = \cos^{-1}(a/R)$. The cross section for γ - γ pair production has a threshold $\epsilon E_\gamma(1 - \cos \beta) = 2m^2c^4$. Choosing the variables $x = \alpha/R$, $s = \epsilon E_\gamma(1 - \cos \beta)/2m^2c^4 = s_0 z$ where $s_0 = \epsilon E_\gamma/2m^2c^4$ and $z = (1 - \cos \beta)$, equation (2) becomes

$$\frac{d\tau}{dR} = \iint 2\pi x^2 n(\epsilon) (1/2x^2 s_0^2) \frac{\sigma(s)}{c} s ds \quad (3)$$

Following the treatment of Gould and Schreder¹⁵ I express ϵ in units of kT and introduce the variable $\nu = 2m^2c^4/(E_\gamma kT z_m)$ where $z_m = (1 - \cos \beta_m)$ and $\beta_m = \sin^{-1}(a/R)$. After performing the integration and rewriting the terms in a manner similar to that of Gould and Schreder

$$\frac{d\tau}{dR} = \frac{\alpha^2}{2\pi\Lambda} \left(\frac{kT}{mc^2}\right)^3 [1 - (1 - x^2)^{1/2}]^2 f(\nu)$$

where $\alpha = e^2/\hbar c$, $\Lambda = \hbar/mc$ and $f(\nu)$ is the function introduced by Gould and Schreder, who give a plot of $f(\nu)$ against ν . The function $f(\nu)$ has a maximum value of ~ 1 at $\nu \simeq 1$. The total optical depth for γ - γ collision is

$$\begin{aligned} \tau_{\gamma\gamma} &= \int \frac{d\tau}{dR} dR = \frac{\alpha^2}{2\pi\Lambda} \left(\frac{kT}{mc^2}\right)^3 a \\ &\int_1^0 [1 - (1 - x^2)^{1/2}]^2 f(\nu) \frac{dx}{x^2} \quad (4) \\ &= 1.2 \times 10^3 T_7^3 I_{\gamma\gamma}(\nu) \quad (5) \end{aligned}$$

where $I_{\gamma\gamma}(\nu)$ is the integral in equation (4), T_7 is the surface temperature in units of 10^7 K and a has been taken as 10^6 cm. I have evaluated $I_{\gamma\gamma}(\nu)$ numerically and Fig. 2 shows a plot of $\tau_{\gamma\gamma} T_7^{-3}$ as a function of $T_7 E_\gamma$ (GeV).

Photodisintegration of heavy nucleus is most effective in the giant resonance region of photon energy 10 to 20 MeV in the rest system of the nucleus. The cross section increases as $A^{2/3}$ where A is the mass number of the nucleus. Here I shall

consider the disintegration of Fe nuclei. The photonuclear cross section can be written as¹⁶

$$\sigma(y) = c(y - 1) \exp[-(b(y - 1))] \quad (6)$$

where b and c are constants and $y = s/p$, p being the threshold energy for the reaction and $s = (\gamma\epsilon)(1 - \cos \beta) s_0 z$ is the energy of the blackbody photon in the rest system of the nucleus of Lorentz factor γ . Going through a similar treatment as for γ - γ collisions and introducing similar variables I arrive at the following expression for $\tau_{\gamma H}$, the optical depth for disintegration of heavy nuclei:

$$\tau_{\gamma H} = 2\pi\sigma_0 \left(\frac{kT}{hc}\right)^3 a I_{\gamma H}(\nu) \quad (7)$$

where

$$I_{\gamma H}(\nu) = \int_1^0 [1 - (1 - x^2)^{1/2}]^2 f_H(\nu) dx/x^2,$$

$$\nu = p/\gamma kT z_m$$

$$f_H(\nu) = \nu^2 \int_\nu^\infty (e^\epsilon - 1)^{-1} \phi(\epsilon/\nu) d\epsilon,$$

$$\phi(y) = \int_1^y y \bar{\sigma}(y) dy, \text{ and}$$

$$\bar{\sigma}(y) = \sigma(y)/\sigma_0 = \sigma(y)/(10^{-25} \text{ cm}^2)$$

For photodisintegration of Fe nuclei, the cross section can be represented as

$$\bar{\sigma}(y) = 11(y - 1) \exp[-2.7(y - 1)]$$

with a threshold of 13 MeV and a maximum cross section of 150 mb at 18 MeV. With this parametrisation for the cross section, the integral $I_{\gamma H}$ has been numerically evaluated and τ_{γ} (Fe) calculated. In Fig. 2 I show the plot of τ_{γ} (Fe T_7^{-3}) as a function of γT_7 .

The total optical depth for γ - γ collision is shown in Fig. 3 for three different values of temperature. For all temperatures $> 2 \times 10^6$ K, there is a wide range of energies in which

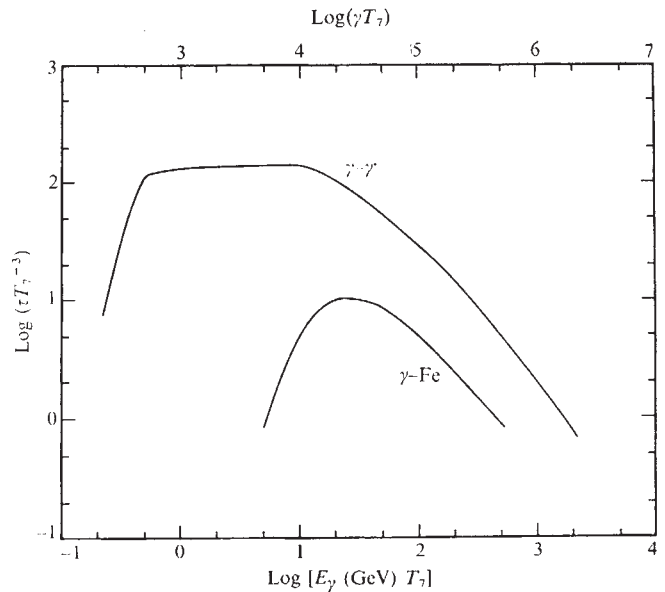


FIG. 2 The quantity $\tau_{\gamma} T_7^{-3}$ plotted as a function of $E_\gamma T_7$ and γT_7 . Here τ is the optical depth for γ - γ and γ -Fe interaction, T_7 is the surface temperature in units of 10^7 K, γ is the Lorentz factor of the Fe nucleus and E_γ the photon energy in GeV.

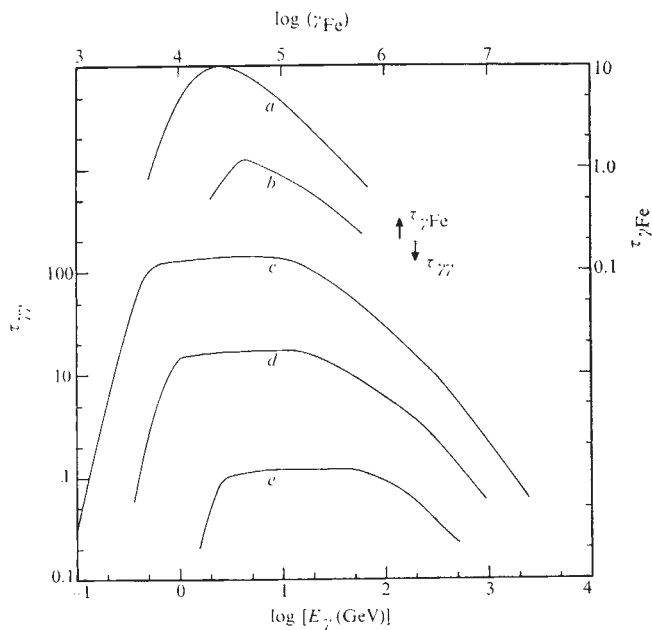


FIG. 3 Optical depth for $\gamma\text{-}\gamma$ interaction (bottom and left scales) and $\gamma\text{-Fe}$ (top and right scales) plotted as a function of energy for different temperatures. Legend as in Fig. 2. a, 10^7 K; b, 5×10^6 K; c, 10^7 K; d, 5×10^6 K; e, 2×10^6 K.

$\tau_{\gamma\gamma} > 1$. The larger the temperature the higher is the optical depth and wider the range of energy for absorption. In the first few hundred years of a pulsar's life, the temperature is greater than 5×10^6 K, leading to the absorption of γ rays of energy ranging from a few hundred MeV to about 1 TeV. When the temperature is 10^7 K the lower limit extends to about 200 MeV. High energy γ rays can also be absorbed because of pair production in the magnetic field of the pulsar. This has been investigated by Sturrock⁴ who finds that for a surface magnetic field of 10^{12} gauss, this process is important for $E\gamma \gtrsim 1$ GeV. The absorption mechanisms discussed in this paper not only extends to lower energies but is also independent of the magnetic field (as well as of the beamed radiation that may be associated with the pulsar).

The optical depth for photodisintegration of Fe nuclei is also shown in Fig. 3 for two different temperatures. Here also the optical depth > 1 for $T > 5 \times 10^6$ K over a reasonably wide range of energies. But the range of energies over which absorption occurs is narrower than that for γ rays. In the first hundred years, the surface temperature of the neutron star is $\sim 10^7$ K. It is during this time that the pulsar loses most of its energy and also accelerates particles up to 10^{18} to 10^{19} eV. The highest energies will not be affected by photodisintegration (Fig. 3); but in the range of a few times 10^{12} eV per nucleon to a few times 10^{14} eV per nucleon, Fe nuclei will be disintegrated preferentially. The medium nuclei will be affected less and over a narrower range of energies, since the photonuclear cross section for them is low by a factor about 2.5. Thus, the cosmic-ray nuclei escaping from the pulsar surface will undergo changes in spectra and composition. Such changes have been observed for cosmic rays with energies of tens of GeV^{17,18}. If a large fraction of cosmic rays come from acceleration processes close to the surface of the neutron star, the above mechanism will induce spectral and compositional changes in the range of 10^{12} eV per nucleon to 10^{14} eV per nucleon.

Smoluchowski¹⁹ has pointed out that because of the anisotropic conductivity of neutron star matter, the surface temperature at the poles can be a factor of 2 to 3 higher than the equatorial temperature. For the absorption processes discussed here, it is the polar temperature which is more

relevant; this being higher than the mean temperature used, the absorption may be more severe than indicated.

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- ¹ Gunn, J. E., and Ostriker, J. P., *Astrophys. J.*, **160**, 979 (1970).
- ² Rees, M. J., *Nature phys. Sci.*, **230**, 55 (1971).
- ³ Komesaroff, M. M., *Nature*, **255**, 612 (1970).
- ⁴ Sturrock, P. A., *Astrophys. J.*, **164**, 529 (1971).
- ⁵ Goldreich, P., and Keeley, D., *Astrophys. J.*, **170**, 463 (1971).
- ⁶ McBreen, B., *Nature*, **224**, 893 (1969).
- ⁷ Rengarajan, T. N., *Proc. eleventh Symposium on Cosmic Rays, Astrophysics, Geophysics and Elementary Particle Physics*, Delhi, India, **II**, 410 (Delhi, 1969).
- ⁸ Stecker, F. W., and Tsuruta, *Nature phys. Sci.*, **235**, 8 (1972).
- ⁹ Apparao, Krishna, and Rengarajan, T. N., *Astrophys. Lett.*, **6**, 229 (1970).
- ¹⁰ Balasubrahmanyam, V. K., Ormes, J. F., and Ryan, M. J., *Proc. int. Cosmic Ray Conf.*, **1**, 344 (Hobart, 1971).
- ¹¹ Rengarajan, T. N., *Proc. int. Cosmic Ray Conf.*, **1**, 350 (Hobart, 1971).
- ¹² Cameron, A. G. W., *A. Rev. Astr. Astrophys.*, **8**, 179 (1970).
- ¹³ Tsuruta, S., Canuto, V., Lodenquai, J., and Rudermann, M., *Astrophys. J.*, **176**, 739 (1972).
- ¹⁴ Rengarajan, T. N., *Nature phys. Sci.*, **242**, 103 (1973).
- ¹⁵ Gould, R. J., and Schreder, G. P., *Phys. Rev.*, **155**, 1404 (1967).
- ¹⁶ Gerasimova, N. M., and Zatsepin, G. J., *Sov. Phys. JETP*, **11**, 399 (1960).
- ¹⁷ Ormes, J. F., and Balasubrahmanyam, V. K., *Nature phys. Sci.*, **241**, 95 (1972).
- ¹⁸ Webber, W. R., Lezniak, J. A., Kish, J. C., and Damle, S. V., *Nature phys. Sci.*, **241**, 96 (1972).
- ¹⁹ Smoluchowski, S., *Nature phys. Sci.*, **240**, 54 (1972).

Duration of dMe flares

GERSHBERG and Shakhovskaya¹ have suggested that the time scales of flare development depend principally on the peak amplitude of flare events, implying that stronger events last longer. They support their contention with observations taken from eleven flare stars. In an earlier investigation of flare decay rates I concluded that time scales are intrinsic properties of the stars themselves².

The relationship determined by Gershberg and Shakhovskaya from their data takes the form

$$\log_{10} \left(-\frac{dL}{dt} \right)_I = 0.75 \log_{10} L_{\text{peak}} + \text{constant} \quad (1)$$

where $(-dL/dt)_I$ is the decay rate on the light curve chosen in a uniform manner and L_{peak} is the peak radiation loss. The corresponding relationship derived by myself, and put in the same form, is

$$\log_{10} \left(-\frac{dL}{dt} \right)_K = \log_{10} L_{\text{peak}} + AM_{\text{bol}} + \text{constant} \quad (2)$$

where M_{bol} is the stellar bolometric absolute magnitude and A is a constant very nearly equal to 0.2.

For any given star the observable range of the magnitude of peak event is constrained by the detection threshold of the instruments used and by flare incidence statistics, so that the less energetic flares are observed preferentially on

TABLE 1 Data sets for determining time scale properties in the flares of UV Ceti

Epoch	Telescope	No. of flares	U_{det}	U_{cuttoff}	No. of flares used
1966.9	91 cm	143	14.6	13.6	25
1967.65	91 cm	38	15.5	14.5	9
1967.77	60 cm	152	14.2	13.2	29
1968.76	91 cm	107	15.6	14.6	35

TABLE 2 Data sets for determining time scale properties in the flares of Wolf 630 and CoD-31°17815

Star	M_V	Epoch	Telescope	Total No. of flares	U_{det}	U_{cutoff}	No. of flares used
Wolf 630	10.8	1968.4	91 cm	24	15.0	14.4	13
		1969.5	60 cm	26	14.6	14.0	10
		1970.5	152 cm	61	15.2	15.0	42
CoD-31° 17815	8.9	1970.5	152 cm	31	15.0	14.8	21

stars of lower luminosity (ref. 3 and Fig. 3 in ref. 1). Because of these effects a biased result is difficult to avoid unless care is taken. Figures 1 and 2 of Gershberg and Shakhovskaya¹ show this effect: the flares of any given star are found in restricted portions of the diagram. Furthermore, because the intrinsic scatter of the parameters which describe flares is large^{2,3}, the relationship presented by these authors could not have been constructed with adequate confidence from observations of a single star. Equation (2) can be made to fit the data of Gershberg and Shakhovskaya as a direct result.

To decide between the alternatives the following two tests are proposed. First, the relationship between peak flare amplitudes and a time scale parameter for the star with the largest homogeneous set of observations is examined. For this purpose my observations^{3,4} of UV Ceti covering the range $9 < U_{peak} < 14.9$ were used. Subtracting $\log_{10} L_{peak}$ from both sides of equation (1), the equation of Gershberg and Shakhovskaya can be written

$$\log_{10} \frac{dU}{dt} = 0.108 U_{peak} + \text{constant} \quad (3)$$

In place of the decay rate, the most densely sampled time scale parameter which I use is the mean duration of half peak light, $T_{0.5}$; other time scale parameters were not measurable for as many of the detected flares. The mean duration and decay rate are inversely related and equation

(3) can therefore be written

$$\log_{10} T_{0.5} = \alpha U_{peak} + \text{constant} \quad (4)$$

where α is -0.108 (ref. 1), or zero (ref. 2).

Table 1 presents the data examined using least squares. The faint cutoff level has been selected 1 mag brighter than the adopted detection limit for the data sets in order to minimise effects of incomplete sampling at the faint event threshold³. Setting U_{cutoff} 0.5 mag fainter altered the value of α by less than one standard error. The values of the coefficient for both instances are

$$\alpha = -0.005 \pm 0.021, \quad 98 \text{ events}$$

$$\alpha = +0.012 \pm 0.025, \quad 132 \text{ events}$$

respectively.

The second test compares the time scales of events of comparable absolute peak energy for stars of different luminosities. Data for Wolf 630 and CoD-31°17815 (ref. 3) suitable for comparison with those of UV Ceti are presented in Table 2. A sufficient range of peak event magnitudes was observed in Wolf 630 to yield a usable value of the coefficient α (-0.012 ± 0.011). As in the case of UV Ceti, there seems to be no significant relationship between the time scales of flares, and peak amplitudes. Because of this, CoD-31°17815 is the most luminous star used in the proposed comparison, even though the observed range of peak magnitudes of events is still too small to allow a reliable determination of the coefficient α .

Figure 1 presents all the data used in this investigation. A correlation analogous to that described by Gershberg and Shakhovskaya is apparent at first glance: the longer time scales are found in the stronger events. But the weakest events detectable for each star depend strongly on stellar luminosity, colour and parallax, and so the effect is deceptive. For the three stars taken together the data are complete only for flares brighter than $M_{V,peak} = 15.06$. If only this portion of the diagram is considered, the apparent correlation disappears completely. Comparing mean time scales of events lying in the range $14.0 < M_{V,peak} < 15.0$, the flares of shortest duration are those of UV Ceti, and the longest lasting are those of CoD-31°17815. The result is shown in Table 3. From these results it is difficult to conclude that the event durations are distinguishable by their peak amplitudes. But they correlate with stellar absolute magnitude as found by Haro and Chavira⁵. A linear relationship describing the effect, is

$$\log_{10} T_{0.5} = 1.084 - 0.119 M_V \quad (5)$$

The analogous equation of Gershberg and Shakhovskaya¹ yields values for $\log_{10} T_{0.5}$ of -0.81 , -0.41 and -0.24 , for UV Ceti, Wolf 630 and CoD-31°17815, respectively.

Gershberg and Shakhovskaya do not explicitly state that their results apply to flare phenomena on isolated stars and indeed the present analysis indicates that no such relationship applies to isolated flare stars: flare durations are not correlated with peak amplitudes. Even if a relationship of the sort proposed by Gershberg and Shakhovskaya had been found, it would still not have precluded a relationship between flare time scales and stellar luminosity: a comparison

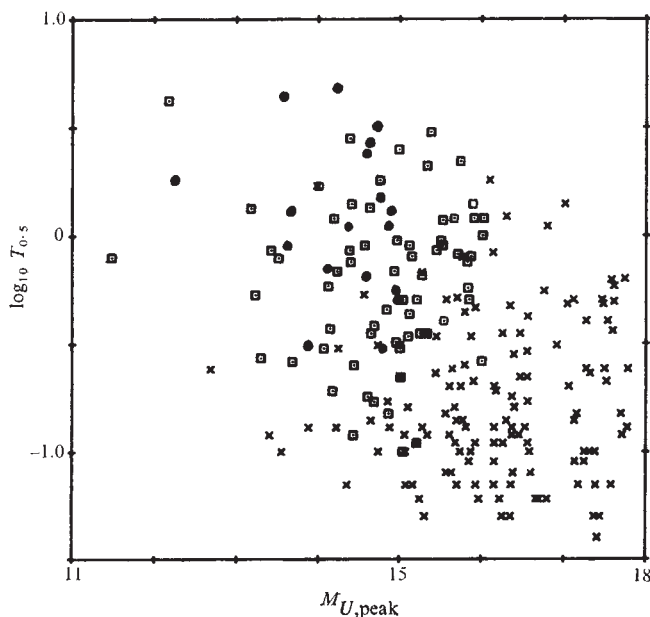


Fig. 1 Relationship between flare durations at half peak light, $T_{0.5}$ (ordinate) and peak light, on a scale of absolute magnitude, $M_{V,peak}$. $\times$, UV Ceti; $\square$, Wolf 630; $\bullet$, CoD-31° 17815. The apparent linear correlation between peak light and mean duration is a selection effect. The data are complete only for events brighter than $M_{V,peak} = 15$. This sector shows no correlation between peak light and event duration.

TABLE 3 Mean values of the event duration parameter $\log_{10} T_{0.5}$ for $14 < M_{V, \text{peak}} < 15$

Star	M_V	$\log_{10} T_{0.5}$	$10^{\log_{10} T_{0.5}}$ (min)	No. of flares
UV Ceti	15.5*	-0.74 ± 0.09	0.184	10
Wolf 630	10.8*	-0.26 ± 0.07	0.552	31
CoD-31 ⁰ 17815	8.9	$+0.07 \pm 0.10$	1.29	13

* Value per component, assuming equal luminosity.

of flares of different stars, which fall in the same range of absolute peak energy levels, shows a strong correlation between the mean event duration and stellar luminosity. In conclusion, it seems that the interpretation of the Haro-Chavira phenomenon proposed by these authors is less appropriate than my interpretation².

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¹ Gershberg, R. E., and Shakhovskaya, N. I., *Nature phys. Sci.*, **242**, 85 (1973).

² Kunkel, W. E., in *Low Luminosity Stars* (edit. by Kumar, S. S.), 195 (1969).

³ Kunkel, W. E., *Astrophys. J. Suppl. Ser.*, **25**, 1 (1973).

⁴ Kunkel, W. E., *Inf. Bull. variable Stars*, 315 (1968).

⁵ Haro, G., and Chavira, E., *Bolletino Tonanzintla y Tacubaya*, **12**, 3 (1955).

Internal origin for lunar rilled craters and the maria?

THE origin of lunar craters has been attributed to both volcanism¹⁻³ and/or impact⁴⁻⁶, although since the return of the Apollo 11 volcanic rocks opinion has favoured an endogenic origin for a greater part of the lunar morphology than was previously accepted⁷.

Evidence for lunar vertical crustal movement is provided by Apollo 15 photographs of dipping structures on Silver Spur (25° 40' N, 3° 58' E)⁸ and by craters containing rilles indicating either uplift⁹ or subsidence¹⁰.

Principal lineaments such as rilles and faults, and craters larger than 10 km containing internal rilles, were drawn on overlays of Orbiter I, II, III, IV and V high and low resolution photographs and transferred to a Mercator projection map (Figs 1 and 2) of the Moon.

Because the morphology of the rilles and their enclosing craters is varied, the internally rilled craters can be divided into a number of subgroups (Fig. 3), by shape and size of the enclosing crater and by the rille pattern on the crater floor.

Small craters are less than 15 km in diameter and the diameter of the floor is less than the rim radius. The large craters are generally greater than 15 km in diameter with the diameter of the floor equal to, or much larger than, the rim radius.

On the near side of the Moon, craters of types I, II and III are situated predominantly around the maria, particularly around the western edge of Oceanus Procellarum, with most situated between the maria and the outer limit of the parallel-sided flat-floored normal rilles (see Figs 1 and 2).

There are too few type IV craters for their distribution pattern to be significant but the type V craters, apart from being found in the vicinity, of type I, II and IV, are also found along the major rifts such as the Rheita Valley. A number are also found within the larger flat-bottomed craters

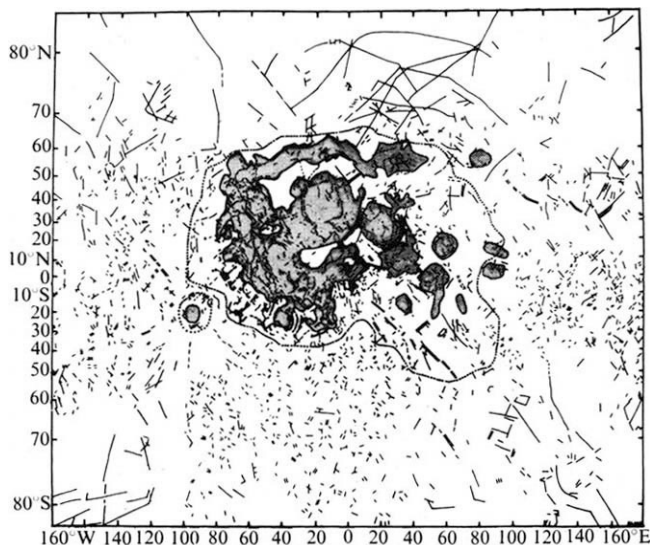


Fig. 1 Map of major lineaments on the Moon; maria have bold outline, dotted line encloses 'normal' rilles.

which themselves contain internal rilles, for example Einstein.

Whereas there are approximately 300 internally rilled craters on the near side of the Moon, there are only about forty on the far side. Although photographic coverage and resolution of the near side is considerably better than that of the far side, photography of the far side is such that only small craters of those occurring in restricted areas of oblique or non-coverage could be added.

A few of the craters on the far side are associated with maria (Komarov) and some are associated with larger craters (Jules Verne and Poincaré groups). The two large craters Oppenheimer and Schrödinger are also rilled. Small rilled craters are again found within larger flat-floored craters (Aitken, Barbier, and Pannekoek). A few internally rilled craters approximately 40 km in diameter (Hogg and Hutton) do not conform to any apparent distribution pattern.

A depth/diameter plot (Fig. 4) of a number of the rilled craters of types I, II and III on the near side (using data from the Arthur catalogue¹¹⁻¹⁴) shows that the majority are wider than the sharply sculptured, well defined, generally deep 'eumorphic' craters which are represented by the line on the diagram (taken from ref. 15).

Because rilles in older craters are much more subdued than those of younger craters, it can be assumed that the rilles in all internally rilled craters formed at about the same time as the crater floor and not as a result of deformation caused by the proximity of the maria.

Figure 4 shows that rilled craters of Copernican, Eratosthenian and pre-Imbrian ages occur in groups approximately parallel to the line for eumorphic craters, with the progressively older craters plotting further toward a smaller depth/diameter ratio. The craters of Imbrian age, however, occur within the fields of all the other groups. This scatter of craters of Imbrian age could be attributed to error in depth/diameter measurements, incorrect identification, or to the fact that the Imbrian craters were produced differently from those of other periods. It is unlikely that the Arthur catalogue is biased toward Imbrian craters alone and the estimated errors for these craters are insufficient to bring the craters into a restricted group on the plot. The Imbrian craters form the basis of the United States Geological Survey lunar stratigraphy so an incorrect age notation is unlikely.

Thus the difference between Imbrian craters and those of other periods is probably due to a peculiarity in the formation of the former. This could have been because vast quantities of lavas were erupted onto the lunar surface during the Imbrian period and the physical constitution of the Moon

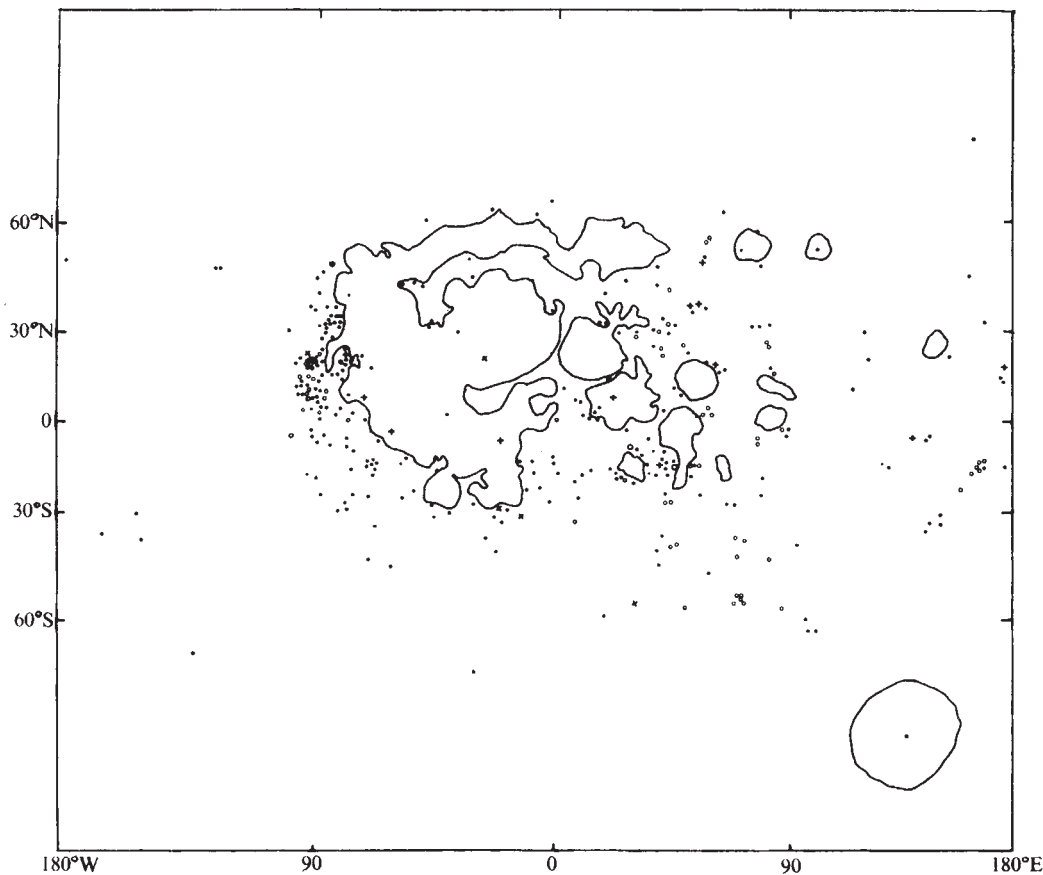


FIG. 2 Distribution map of rilled craters on the Moon; ●, type I; ■, type II; X, type III; +, type IV; ○, type V.

at this time may have been radically different from that of earlier and later periods.

Another problem associated with the origin of the rilled craters is that they occur adjacent to grossly similar craters without rilles. Possible explanations for this are that either the rilles in non-rilled craters are covered by later deposits, or that structural defects in the crust beneath rilled craters may have caused preferential rille development. Counts of craters larger than 1 mm within both rilled and non-rilled craters were carried out on the high resolution Orbiter photographs. The results (Fig. 5) show that in most cases the floors of the non-rilled craters have a higher crater density than the floors of the rilled craters in the same age group, arguing against the possibility of a younger floor covering the rilles. The greater crater density may, however, be due to a high percentage of small endogenic craters.

The simplest explanation for rilled and non-rilled craters is that they have tectonically different origins. If the craters are of impact origin, the rilled craters may have been deeper, or have formed in structurally weaker material which allowed a greater degree of isostatic readjustment after infilling, producing the rilles. If, on the other hand, the craters are of endogenic origin the rilles could have been produced by

either updoming of a diapiric body or subsidence after the evacuation of a magma chamber.

The geographical distribution (Fig. 2) of types I, II and III rilled craters indicates that their formation was also

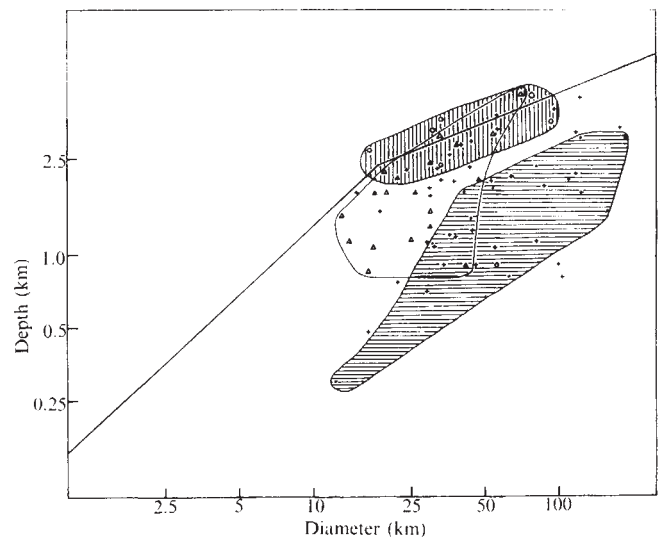


FIG. 4 Depth/diameter graphs of some rilled craters on the near side; horizontal ruling, pre-Imbrian; vertical ruling, Copernican; unshaded outline, Eratosthenian; ○, Copernican; ▲, Eratosthenian; +, Imbrian; ●, pre-Imbrian.

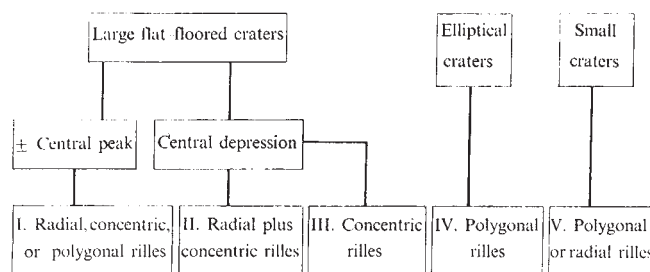


FIG. 3 Classification of rilled craters.

related to the maria. A number of hypotheses can be advanced to account for this relationship depending on whether: (a) both the internally rilled craters and mare basins are of external origin; (b) the craters are of internal origin and the mare basins were formed by impact; (c) the mare basins are of internal origin and the craters were formed by impact; (d) they are both of internal origin.

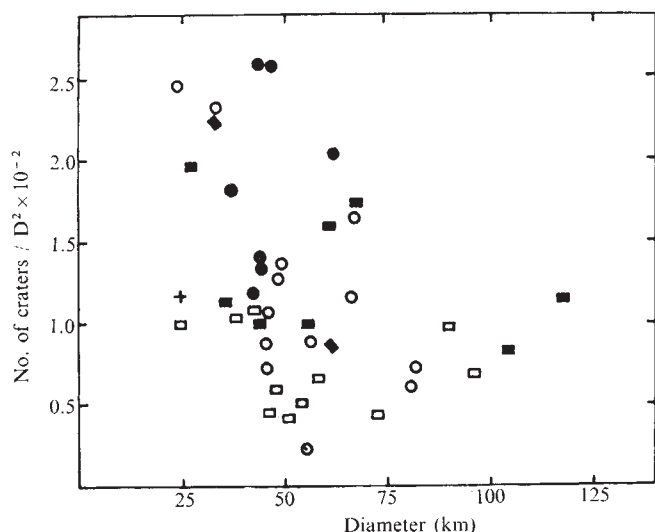


FIG. 5 Crater density plot for small craters inside rilled and non-rilled craters. Subdivided on age taken from refs 11-14. Rilled: ● = 3; ○ = 2; ◐ = 1. Non-rilled: ◆ = 4; ■ = 3; □ = 2; + = 1. D, Diameter.

(a) If the rilles within pre-Imbrian rilled craters are of pre-Imbrian age, this first hypothesis is unlikely since bodies would have to have struck the Moon during the pre-Imbrian period within the limits of the then non-existent normal rilles. (These impacts would have been followed by much larger impacts forming the mare basins within the area defined by the pre-Imbrian impacts but not covering such a large geographical area of the Moon. These in turn were followed by smaller bodies which were preserved immediately around the edges of the maria but became swallowed up in the maria. The whole argument sounds very unconvincing.)

(b) If it is argued that the rilled craters are of internal origin as a result of their proximity to the maria, the presence of pre-Imbrian rilled craters would be related to, and surround, an impact site that did not yet exist.

(c) Arguments against this hypothesis are similar to those of (a) and (b) in that it is very difficult to explain the observed crater distributions.

(d) If the mare basins are of internal origin, the distribution of the rilled craters can be readily explained as resulting from their situation in areas of lunar crustal weakness which on the near side of the Moon were centred on the maria. Internal magmatic processes originating in the pre-Imbrian period reached a maximum during the Imbrian period with the formation of the maria by lava eruption and block subsidence. This internal activity steadily declined and became confined to the areas surrounding the maria where normal rilles, mare terraces and rilled craters formed as a result of crustal uplift and subsidence. The differences between the Imbrian rilled craters and those of other periods could be a result of the peculiar state of the lunar crust during the outpouring the mare lavas.

The rilled craters on the far side of the Moon can be similarly interpreted as occurring in areas of lunar crustal weakness. Because the crust is thicker on that side of the Moon¹⁶, either the magma supply was insufficient or magma could not be supplied to the surface at sufficient speeds to produce extensive maria.

It is not necessary to propose an impact origin to account for the areas of weak crust. Structural effects, such as fracturing and faulting, associated with the formation of the lunar grid of Moonwide extent (ref. 2, and G. Fielder and J. L. Whitford-Stark, to be published) could have produced these weaknesses.

The rilled structures in the types IV and V craters could be of volcanic origin; either by cooling contraction of a lava

lake or tholoid doming. Other evidence for their being of internal origin is that many are situated along probable faults, such as the Rheita Valley.

To summarise, if the rilles in large rilled craters are the same age as the enclosing crater, the distribution of the craters precludes them from being anything other than of internal origin and, as a consequence, the maria and their basins must also be of internal origin. The types IV and V craters are also probably related to volcanic processes.

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- Spurr, J. E., *Features of the Moon*, (Science Press Printing Co., Lancaster, Pennsylvania, 1944).
- Fielder, G., *Lunar Geology*, (Lutterworth Press, London, 1965).
- Green, J., *J. geophys. Res.*, **76**, 5719-5731 (1971).
- Baldwin, R. B., *The Measure of the Moon*, (University of Chicago Press, Chicago, 1963).
- Öpik, E. J., *A. Rev. Astr. Astrophys.*, **7**, 473 (1969).
- Shoemaker, E. M., in *Physics & Astronomy of the Moon*, (edit. by Kopal, Z.), (Academic Press, London, 1962).
- El-Baz, F., *Lunar Science 4*, (edit. by Chamberlain, J. W., and Watkins, C.) 215 (1973).
- Wolfe, E. W., and Bailey, N. G., *Proc. third lunar Sci. Conf. (Suppl. 3 Geochim. cosmochim. Acta)*, **1**, 15-25 (1972).
- Baldwin, R. B., *J. geophys. Res.*, **73**, 3227-3229 (1968).
- De Hon, R. A., *J. geophys. Res.*, **76**, 5712-5718 (1971).
- Arthur, D. W. G., Agnieray, A. P., Horvath, R. A., Wood, C. A., and Chapman, C. R., *Comm. Lunar and Planetary Lab., Univ. Arizona*, **2**, 71-78 (1963).
- Arthur, D. W. G., Agnieray, A. P., Horvath, R. A., Wood, C. A., and Chapman, C. R., *Comm. Lunar and Planetary Lab., Univ. Arizona*, **3**, 1-59 (1964).
- Arthur, D. W. G., Agnieray, A. P., Pellicori, R. H., Wood, C. A., and Weller, T., *Comm. Lunar and Planetary Lab., Univ. Arizona*, **3**, 61-146 (1965).
- Arthur, D. W. G., Pellicori, R. H., and Wood, C. A., *Comm. Lunar and Planetary Lab., Univ. Arizona*, **5**, 1-208 (1966).
- Pike, R. J., *J. geophys. Res.*, **72**, 2099-2106 (1967).
- Beals, C. S., *J. geophys. Res.*, **76**, 5586-5595 (1971).

Photo-induced free radicals on a simulated Martian surface

THE atmospheric pressure at the surface of Mars is about 5.5 mbar and the atmosphere is composed mainly of CO₂ with trace amounts of CO, O₂ and H₂O (refs 1 and 2). The Martian dust has an intermediate SiO₂ content of 60 ± 10% (ref. 3). Although it has been thought that the synthesis in a planetary atmosphere of organic compounds relevant to the origin of life requires chemically reducing conditions, Hubbard and colleagues^{4,5} have reported the synthesis of organic compounds by ultraviolet irradiation ($\lambda > 250$ nm) of a solid with a gas mixture adsorbed which is compositionally similar to the oxidised atmosphere of Mars. Since the question whether organic compounds could have been formed on the surface of Mars is of fundamental importance to the concept of chemical evolution and to the interpretation of results from the future Mars Viking Mission molecular analysis experiment, we have attempted to confirm these results while concentrating on identifying the primary process and the reactive intermediates involved. Here we describe the electron spin resonance (ESR) study of free radicals in the ultraviolet irradiation of a simulated Martian surface. (A preliminary account was presented at the Fourth International Conference on the Origin of Life, Barcelona, Spain, June 1973.)

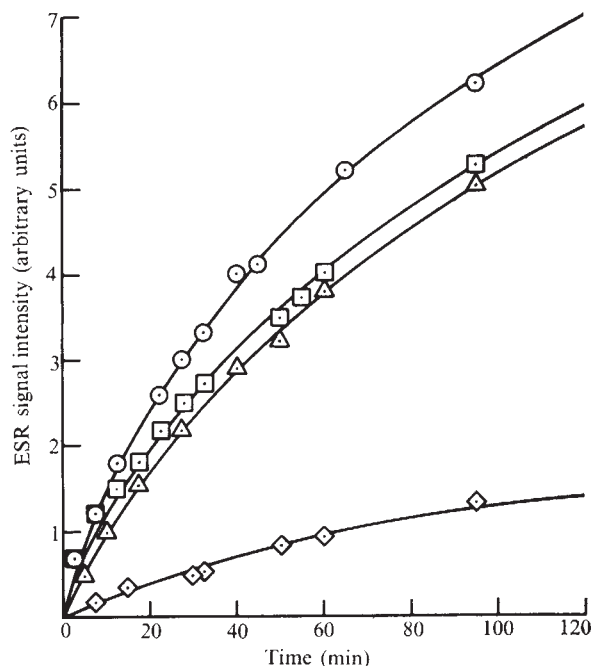


Fig. 1 Formation of CO_2H radicals produced at -170°C by ultraviolet irradiation of CO (15 Torr), CO_2 (15 mmHg), CO (15 mmHg) + CO_2 (135 mmHg), and CO (15 mmHg) + CO_2 (135 mmHg) + O_2 (1 mmHg) adsorbed on silica gel. ○, CO; □, CO + CO_2 ; △, CO + CO_2 + O_2 ; ◇, CO_2 .

The silica gel used in these experiments consisted of 60 to 80 mesh, acid-washed gas chromatograph grade powder. The CO (free of iron carbonyl), CO_2 and O_2 used were Matheson high quality research grade. The vacuum system used to introduce gases had had no previous exposure to mercury. In a typical experiment, the silica gel (200 mg) was placed in an ESR tube (made of Suprasil, 20×0.4 cm outer diameter) and was heated at 180°C for 6 h in a vacuum of 10^{-5} mmHg, then continuously evacuated for 16 h at room temperature. Various gases were then introduced through the vacuum line into the ESR tube and exposed to the silica surfaces. The resultant sample, in the sealed ESR tube, was then placed in an ESR cavity and irradiated with a low pressure mercury lamp through a Vycor filter. Thus the ultraviolet light used for irradiations contained, chiefly, the 253.7 nm line with minor components in the longer wavelength region. Irradiation was usually carried out at -170°C . The ESR spectra were recorded on a Varian V-4500 X-band spectrometer equipped with a variable temperature attachment. The g values were obtained by comparison with the standard value for diphenylpicryl hydrazyl (DPPH, $g = 2.0037$). The estimated error for each g value was about ± 0.0002 . The vapour pressures for all gases indicated below were those observed at room temperature with a calibrated thermal conductivity gauge. These values were arbitrarily chosen for experimental convenience; nevertheless, CO_2 was kept in excess in the systems containing a mixture of gases.

Irradiation of the silica gel alone at -170°C slowly produced a radical species, as evidenced by the appearance of the ESR signal. The spectrum consists of a major peak with $g = 2.0120$ and a minor peak at a field 27 gauss lower ($g = 2.0292$). The g value of the major peak is consistent with the reported g value (2.01) for the OH radical produced in a frozen H_2O medium at -196°C (ref. 6). The same ESR spectrum was obtained more quickly when vapour from dilute aqueous H_2O_2 (vapour pressure, 0.3 mmHg) adsorbed on silica gel was irradiated at -170°C . Both radicals showed the same thermal decay behaviour; the ESR signals disappeared when the samples were warmed to room temperature. Since photolysis of H_2O_2 with 253.7 nm light results only in OH formation⁷, we conclude that this major peak is due

to the OH radical. Exposure of the silica gel to H_2O vapour (5 mmHg) followed by irradiation at -170°C did not lead to enhanced signal intensity due to additional OH radical formation.

When the silica gel was exposed to O_2 (1 mmHg) and then irradiated at -170°C , a new radical species was formed. The spectrum shows g value anisotropy having $g_x = 2.0255$, $g_y = 2.0088$, and $g_z = 2.0037$. It also shows doublet hyperfine splitting of 10 gauss for the peak at g_x because of coupling to a proton. This spectrum is identical with that of the O_2H radical reported by Smith and Wyard⁸.

When a silica gel sample containing CO (15 mmHg), or CO_2 (15 mmHg), or a mixture of CO (15 mmHg) and CO_2 (135 mmHg) was irradiated at -170°C , a different radical was observed. The signal appears at a higher field than the OH radical, and the line shape shows characteristic g -value anisotropy. The g values for this new radical are $g_x = g_y = 2.0024$ and $g_z = 1.9971$, and its isotropic or average g value is 2.0006, which is close to the isotropic liquid g value of 2.0002 reported for the CO_2H radical⁹, and which is in excellent agreement with the average g -value of 2.0005 attributed by Chantry *et al.*¹⁰ to the CO_2H radical formed by gamma irradiation of potassium bicarbonate crystals. Furthermore, the average g value (2.0006) of this radical is below the free spin value (2.0023), indicating that it is unlikely to be the π radical, HCO_2 . But it is consistent with the σ radical, CO_2H , in which the unpaired spin resides on an orbital of approximately sp^2 type¹¹. The g anisotropy of the CO_2H radical indicates that it was formed on the silica surface where it was stable for hours in the dark at -170°C . On warming it decayed rapidly and the ESR spectrum disappeared at about -50°C . The CO_2H radical was the only radical product observed at -170°C when a mixture of O_2 (1 mmHg), CO (15 mmHg), and CO_2 (135 mmHg) adsorbed on silica gel was irradiated. The relative yields of the CO_2H radical obtained under various conditions as estimated from ESR signal intensity are shown in Fig. 1. No ESR signal was observed when CO, CO + H_2O , CO + CO_2 , or CO + CO_2 + O_2 was irradiated at -170°C in the absence of silica gel.

After warming to room temperature, continued irradiation of all previously irradiated samples (CO, CO_2 , CO + CO_2 or CO + CO_2 + O_2 adsorbed on silica gel) resulted in the generation of a third radical species. The line width is narrower (3 gauss, compared with 5 gauss for CO_2H). Furthermore, this radical was stable at room temperature for days, thus, indicating that a new radical was formed. The g values for this radical are $g_x = g_y = 2.0024$ and $g_z = 1.9979$. Comparison of the ESR line shape, line width and g values of this radical with other reported data^{12,13} reveals that this is the carbon dioxide anion radical, CO_2^- . When HCO_2H was adsorbed on silica gel and irradiated at room temperature, an identical ESR spectrum was obtained. These two radicals also show the same thermal decay behaviour.

Results of the present ESR study suggest that the ultraviolet photolysis of CO or CO_2 , or a mixture of CO and CO_2 adsorbed on silica gel at -170°C , involves the formation of OH radicals and possibly H atoms as the primary process, followed by the formation of CO_2H radicals. Although other radicals might also have been formed, microwave power saturation and thermal decay experiments show that the indicated radicals were the principal radical products. The H atoms and OH radicals may be produced by: (i) direct photodissociation of H_2O vapour and/or H_2O physically adsorbed on silica gel; (ii) absorption of the ultraviolet excitation energy by the silica surface with subsequent cleavage of the silanol bonds, ($=\text{Si}-\text{H}$ and $=\text{Si}-\text{OH}$); (iii) energy transfer from silica gel to adsorbed H_2O followed by cleavage of $\text{H}-\text{OH}$ bond; or (iv) a combination of the above. Process (i) seems very unlikely since ultraviolet irradiation was carried out with the wavelength (>253.7 nm) longer than that absorbed by H_2O molecules. Moreover, no ESR signal was observed when H_2O ad-

sorbed on NaCl was irradiated at -170°C with the same light source. In the NaCl system, only H_2O vapour and physically adsorbed H_2O were present. Furthermore, no ESR signal was observed when a mixture of CO (15 mmHg), CO_2 (135 mmHg) and H_2O (1 mmHg) adsorbed on NaCl was irradiated at -170°C . We therefore conclude that either (ii) or (iii) or (iv) is more likely. Similar processes such as photosensitisation of hydrogen dissociation by silica gel¹⁴, and ultraviolet-induced bond cleavages of =Si-OCH_3 and =Si-CH_3 (ref. 15) have been reported. The energy transfer process (iii) may be of minor importance relative to cleavage of silanol bonds (ii) since there was no significant increase in the ESR signal due to OH radicals during the irradiation of silica gel containing 5 mmHg of H_2O vapour. When silica gel preheated at 450°C (instead of 150°C) was irradiated at -170°C , only a very weak ESR signal of the OH radical was observed. Apparently, at this temperature, most of the silanol groups condensed to liberate H_2O , leaving surface siloxane groups¹⁶.

H atoms were not observed by ESR in our experiments, possibly because of their low concentration and/or rapid combination to form H_2 molecules. Reactions of the H atom and OH radical with CO can form the HCO radical and CO_2H radical, respectively. CO is an efficient scavenger for the OH radical¹⁷; therefore, the formation of CO_2H radical is expected to be favoured. Presumably because of the low concentration of free H atoms available for capture by CO, the CHO radical could not be detected under the conditions of these experiments. In fact, Hubbard *et al.*^{4,5} observed only a very low yield of HCHO as compared to HCO_2H . When CO_2 is adsorbed on silica gel, photochemically produced H atoms could react with CO_2 and reduce it to CO_2H . But reaction of OH radicals with trace amounts of CO present in CO_2 as impurity might also contribute to the formation of CO_2H radicals. For the mixture of CO and CO_2 on silica gel, both gases were adsorbed on the surface, so the sites available for CO adsorption would be decreased. Consequently, the total amounts of CO_2H radical formed would be lower than when CO alone was adsorbed on silica gel (Fig. 1).

The formation of HCO_2H from CO_2H radicals was demonstrated by the irradiation experiment at room temperature. After the CO_2H radicals had disappeared upon warming, re-irradiation of the silica gel sample at room temperature produced CO_2^- radicals. This same radical was also observed when authentic HCO_2H adsorbed on silica gel was irradiated at room temperature. Apparently, HCO_2H was formed during the thermal decay of CO_2H radicals, and irradiation at room temperature resulted in the photodecomposition of HCO_2H .

This study confirms the reports of Hubbard *et al.* that HCO_2H and possibly HCHO and other organic compounds were formed during the photolysis of atmospheric gases adsorbed on a simulated Martian surface, suggesting that these compounds may be formed on the surface of Mars as well. An important observation in this study is that these organic compounds were formed by the reaction of free radicals generated by the ultraviolet irradiation, with OH radicals playing a very important role. This observation is in contrast to the mechanism proposed by Hubbard *et al.*, who suggest that CO excitation is responsible for the organic synthesis. Our study suggests that photochemical synthesis of organic compounds could occur on Mars if the siliceous surface dust contains enough silanol groups and/or adsorbed H_2O , as bound water. Houck *et al.*¹⁸ recently provided high altitude infrared spectroscopic evidence that the wind-blown surface dust on Mars contains about 1% bound water by weight. The low partial pressure of CO in the Martian atmosphere probably would not pose serious problems to the organic synthesis since CO_2 , which is present in large quantity, also could react with H atoms to form CO_2H radicals. The effect of O_2 on these syntheses is minimum, since in our ex-

periment the addition of 1 mmHg of O_2 to the CO-CO_2 mixture adsorbed on silica gel showed little effect on the CO_2H radical yield (Fig. 1). We suggest that further study of such heterogeneous systems may provide a mechanistic basis for gauging the potential importance of gas-solid photochemistry for chemical evolution on other extraterrestrial bodies, on the primitive Earth, and on dust grains in the interstellar medium¹⁹.

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- 1 Carleton, N. P., and Traub, W. A., *Science*, **177**, 988 (1972).
- 2 Barker, E. S., *Nature*, **238**, 447 (1972).
- 3 Hanel, R., Hovis, C. W., Kunde, V., Lowman, P., Maguire, W., Pearl, J., Pirraglia, J., Prabhakara, C., Schlachman, B., Levin, G., Straat, P., and Burke, T., *Icarus*, **17**, 423 (1972).
- 4 Hubbard, J. S., Hardy, J. P., and Horwitz, N. H., *Proc. natn. Acad. Sci. USA*, **68**, 574 (1971).
- 5 Hubbard, J. S., Hardy, J. P., Voecks, G. E., and Golub, E. E., *J. molec. Evolution*, **2**, 149 (1973).
- 6 Kroh, J., Green, B. C., and Spinks, J. W. T., *Can. J. Chem.*, **40**, 413 (1962).
- 7 Calvert, J. G., and Pitts, J. N., *Photochemistry* **202** (Wiley, New York, 1966).
- 8 Smith, R. C., and Wyard, S. J., *Fifth International Symposium of Free Radicals*, 66-2 (Gordon and Breach, New York, 1961).
- 9 Norman, R. O. C., and West, P. R., *J. chem. Soc. (B)*, 389 (1969).
- 10 Chantry, G. W., Horsfield, A., Morton, J. R., and Whiffen, D. H., *Molec. Phys.*, **5**, 589 (1962).
- 11 Wertz, J. E., and Bolton, J. R., *Electron Spin Resonance—Elementary Theory and Practical Applications*, 174 (McGraw-Hill, New York, 1972).
- 12 Miura, M., Hasegawa, A., and Yamada, Y., *Bull. chem. Soc. Japan*, **41**, 1320 (1968).
- 13 Beckwith, A. L. J., and Norman, R. O. C., *J. chem. Soc. (B)*, 400 (1969).
- 14 Pariiskii, G. B., Mikheikin, I. D., Rez, A. I., and Kazanskii, V. B., *Dokl. Akad. Nauk. SSSR*, **180**, 123 (1968).
- 15 Melamud, G., Reisner, M. G., and Garbatski, U., *J. phys. Chem.*, **77**, 1023 (1973).
- 16 Snyder, L. R., *Principles of Adsorption Chromatography*, 155 (Marcel Dekker, New York, 1968).
- 17 Buxton, G., and Wilmarth, W. V., *J. phys. Chem.*, **67**, 2835 (1963).
- 18 Houck, J. R., Pollack, J. B., Sagan, C., Schaack, D., and Decker, J. A., jun., *Icarus*, **18**, 470 (1973).
- 19 Breuer, H. D., *Molecules in The Galactic Environment* (edit. by Gordon, M. A., and Snyder, L. E.), 381 (Wiley, New York, 1973).

Microearthquake survey of Median Valley of the Mid-Atlantic ridge at $36^{\circ}30'\text{N}$

MICROEARTHQUAKE activity in the median valley of the Mid-Atlantic Ridge near $36^{\circ}30'\text{N}$ has been monitored using expendable radio-sonobuoy arrays. Similar techniques have been used by Reid *et al.*¹ in the Gulf of California, Macdonald and Mudie² near the Galapagos spreading centre and recently by Reid and Macdonald³ near fracture zones A and B shown in Fig. 1 (ref. 4). This study provides area coverage not obtained by Reid and Macdonald whose experiment was confined to the fracture zone regions. A new method of sonobuoy position determination was used that provides relative buoy locations (locations with respect to each other) to within ± 10 m. The ultimate positioning capability in latitude and longitude depends on the accuracy of the primary navigation system available. Satellite navigation can yield absolute positions to within ± 100 m. An estimated actual accuracy of ± 200 m is ascribed to the experiments



FIG. 1 Bathymetric map of part of the Mid-Atlantic Ridge. Epicentre locations obtained in this study are shown as large circles. Small circles represent epicentres determined by Reid and Macdonald³. Approximate location of fracture zones and median valley is shown. Contours are in uncorrected fathoms.

reported here. Accuracy of this order allows correlation of epicentre locations with topographic features of similar scale size. Recently acquired bathymetric data (K. C. Macdonald and colleagues, in preparation) indicate that significant features of the ridge near 36°30'N, such as transform faults and valley walls, can be defined by scale sizes of some hundreds of metres. A total of 112 events definitely attributable to seismic activity were recorded in 72 h of listening, yielding an average rate of occurrence of 1.5 events h^{-1} .

Arrays consisting of three US Navy AN/SSQ-57 calibrated sonobuoys, with 91.4-m hydrophone depth, were launched at three locations within the study area. The arrays approximated equilateral triangles with legs of 1.5 to 2 km. Sonobuoy positions were continuously monitored in real time by an acoustic navigation system originally designed to track the course of the submersible DSRV Alvin, but modified to track a moving hydrophone. The system uses either two or three bottom-moored acoustic transponders and a shipboard transmitter/receiver. (Two transponders make orientation with respect to the transponder baseline ambiguous; three transponders resolve the ambiguity. Often the ambiguity can be resolved from bathymetric data, or alternative navigation

aides, thus allowing the use of only two transponders.) Determination of the position of a sonobuoy consists of first measuring the round trip travel time of an acoustic pulse emitted by the ship, received and retransmitted at a different frequency by each transponder and received aboard ship. This observation provides the one way travel time from ship to transponder, t_1 . A second transmitted pulse is received by the transponders and retransmitted, but this time is received by the sonobuoy, thus determining the ship-transponder-sonobuoy acoustic travel time, t_2 . The travel time from transponders to buoy is $t_1 - t_2$. Slant ranges to the transponders are computed, corrections are made for sound velocity variation with depth (ray bending), and buoy position is resolved into a rectangular coordinate system whose origin is fixed with respect to the transponder net. Travel times can be determined to within 2 ms, thus establishing a limitation of about ± 3 m in buoy positioning. In practice a sonobuoy fix can be obtained every 20 to 30 s. (The maximum round-trip travel time range of the navigation system was about 12 s.) Absolute positioning of the transponder net in latitude and longitude is done using satellite fixes.

Seismic signals from the sonobuoy array elements were low pass filtered (0 to 500 Hz) and recorded on an f.m. analogue tape recorder with frequency response $+0$, -0.7 dB from d.c. to 5 kHz. An example of three events received by a single sonobuoy within an interval of about 40 min is shown in Fig. 2. The event depicted in Fig. 2a occurred within the bounds of the triangular array, less than 1 km away from all sonobuoys. First compressional wave arrival (P) and two reflections of that wave through the water column (P_1 and P_2) are clearly shown. Shear waves (S) and direct water waves (T) are not evident because of the proximity of the event. Figures 2b and 2c are more distant and exhibit prominent T-phase arrivals. These figures give an indication of the excellent recording conditions encountered. Sea states were typically 0 to 1/2 with wind and swell near zero. Ambient noise levels at 25 Hz were chiefly less than -10 dB with respect to $1 \mu\text{bar}$. It is estimated that the array could detect microearthquakes of magnitude $M > -1$.

Power spectra of the events shown in Fig. 2 are shown in Fig. 3. These spectra have been corrected for the rising frequency response of the calibrated sonobuoys and represent an earthquake signal in excess of background noise. The spectra peak is about 15 to 25 Hz, rather greater than the typical peak frequencies of distant events⁵. This is not surprising since there is increased attenuation of high frequencies over long propagation paths. Measured peak pressures are

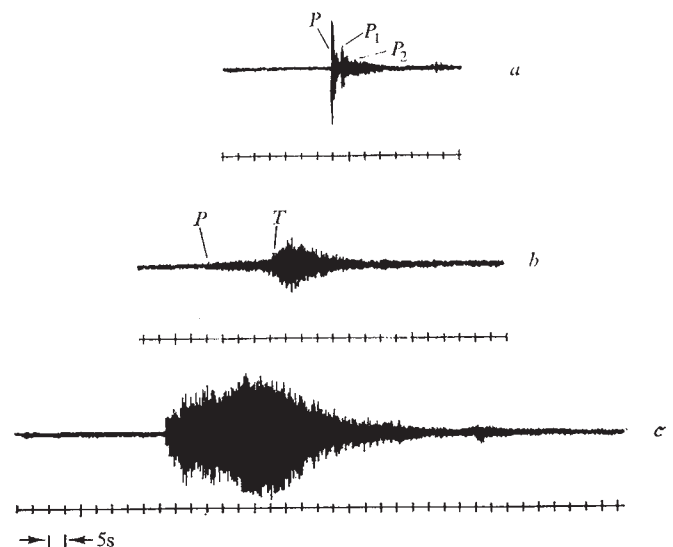


FIG. 2 Example of microearthquakes received with a single sonobuoy. Event *a* is less than 1 km horizontal range from the receiving hydrophone. *a*, 0231:30; *b*, 0156:40; *c*, 0154.

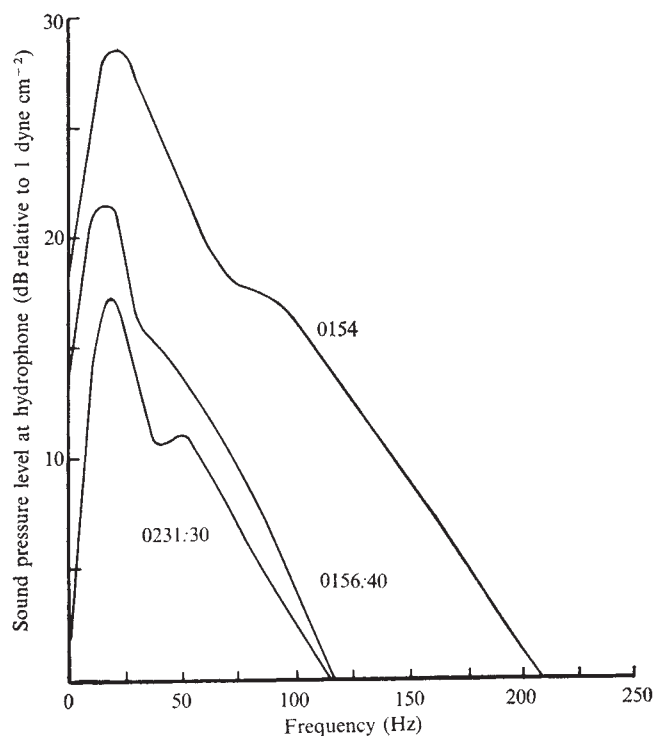


FIG. 3 Power spectra of three typical events (signal in excess of noise).

comparable to an equivalent source strength of some 75 to 100 dB relative to 1 μ bar at 1 m assuming spherical spreading over the entire transmission path.

Earthquake epicentre locations were determined by measuring the relative arrival times of the initial P wave on all three buoys. A horizontal, plane bottom was assumed, characterized by a compressional velocity of 5,000 ms^{-1} . When S-wave arrivals were distinct, S-P travel time differences were also used. The arrival times were solved iteratively for epicentre location using a computer. Geographical location accuracy is estimated to be within ± 200 m, the major sources of error being the estimate of absolute latitude and longitude of the transponder navigation net, the assumed crustal compressional velocity of 5,000 m s^{-1} and the assumption of zero focal depth^{3,6}.

Although 112 events were recorded, only 29 had sufficiently distinct arrival times to allow accurate epicentre location. The small array dimensions (restricted by the range of the acoustic navigation net) made it impossible to locate events more than about 20 km away from the array. Thus it is difficult to compare the level of seismicity in the median valley with that near transform faults because virtually all recording time was over median valley segments. Reid and Macdonald measured about 0.5 events h^{-1} on fracture zones A and B, while we measured 1.5 events h^{-1} , but their system was less sensitive because of poor weather conditions and higher ambient noise levels.

All but two of the located events occurred along the boundary between the median valley floor and the median valley wall. Near-bottom, bathymetric profiles in the vicinity of the median valley both north and south of fracture zone B show that the median valley walls are constructed predominantly of normal faults (K. C. Macdonald *et al.*, unpublished). Since the microearthquakes in this study cluster at the edges of the median valley and not along the axis, the tremors are probably associated with the incipient and ongoing uplift of fault blocks forming the wall rather than with the dynamics of intrusion in the centre of the median valley. Indeed, the events located occurred only along the eastern edge of the median valley where the east rift wall

begins, even though the array detection range was sufficient to detect events along the west wall and valley axis. This asymmetry is also apparent in the bathymetry and magnetics; the regional gradient of the rift walls is more gradual on the east side, and the axis of the central magnetic anomaly is offset several kilometres to the east (ref. 5 and H. D. Needham and F. Francheteau, to be published). Although our sampling is over a very short time interval, the seismicity distribution suggests that seafloor spreading activity here may be highly asymmetric, skewed towards the east at present.

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¹ Reid, I., Reichle, M., Brune, J., and Bradner, H., *Geophys. J. R. astr. Soc.*, (in the press).

² Macdonald, K. C., and Mudie, J. D., *Geophys. J. R. astr. Soc.*, (in the press).

³ Reid, I., and Macdonald, K. C., *Nature*, (in the press).

⁴ Phillips, J. D., *Trans. Am. geophys. Union*, **54**, 242 (1973).

⁵ Milne, A. R., *Bull. seism. Soc. Am.*, **49**, 317 (1959).

⁶ Weidner, D. J., and Aki, Keiiti, *J. geophys. Res.*, **78**, 1818 (1973).

Velocity ratios for rocks with oriented microcracks

THE velocity of sound in 55 gneisses from the Alps has been determined by Johnson and Wenk¹ in ambient conditions. Because of their foliated fabric, these rocks show a strong anisotropy in physical properties chiefly on account of preferentially oriented microcracks at the boundaries of mineral grains. Johnson and Wenk's determinations were made along the principal fabric directions, assuming orthorhombic symmetry; following their notation, the *c* direction is perpendicular to the foliation and therefore to the oriented microcracks, and the *a* and *b* directions lie within the plane of foliation. Along each direction of wave propagation, there are two shear velocities, corresponding to the two mutually perpendicular directions of polarisation parallel to the principal axes.

We analysed the data provided by Johnson and Wenk and found that, at 95% confidence interval, the mean values of the ratios between the velocities of the compressional waves and shear waves are: along the *a* direction, $\alpha_a/\beta_{ab} = 1.42 \pm 0.02$ and $\alpha_a/\beta_{ac} = 1.65 \pm 0.03$; along the *b* direction, $\alpha_b/\beta_{ba} = 1.59 \pm 0.02$ and $\alpha_b/\beta_{bc} = 1.77 \pm 0.03$; and along the *c* direction, $\alpha_c/\beta_{ca} = 1.29 \pm 0.02$ and $\alpha_c/\beta_{cb} = 1.26 \pm 0.02$. Thus there is distinctive anisotropy in the velocity ratios for the *a* and *b* directions where the polarisation of shear waves is either perpendicular or parallel to the foliation. On the other hand, along the *c* direction, where the polarised shear waves all lie within the plane of foliation, there is little anisotropy in the velocity ratio. A more interesting result is that the ratios for the *c* direction are very much

lower than those along the other directions, apparently because of the oriented microcracks perpendicular to the direction of wave propagation. These observations are in qualitative agreement with the theoretical prediction by Anderson *et al.*².

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¹ Johnson, L. R., and Wenk, H.-R., *Tectonophysics* (in the press).

² Anderson, D. L., Minster, B., and Cole, D., *J. geophys. Res.* (in the press).

Surface energy calculations for muscovite

MUSCOVITE, $\text{KAl}_2(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH})_2$, has cleavage properties which make it suitable for studying phenomena which occur on surfaces and interfaces. The excellent cleavage produces surfaces which will readhere with the same or nearly the same strength¹⁻³, and which can be molecularly smooth over large areas⁴. Experimental results indicate that the attraction across the (001) cleavage is greatest in a vacuum and becomes less in air or various other media, and there is evidence that the rate of cleavage influences the strength of attraction⁵.

Theoretical studies have been based on the assumption that electrostatic forces provide the major component of the interlayer attraction and this is supported by the observation that cleaving mica creates electrical charges on the cleavage surfaces. Computations depend on a knowledge of the arrangement of the atoms on the surface of the cleavage plane, known ionic charges, and the distances to ions on the other side of the cleavage plane. Splitting occurs along the plane containing the potassium ions, which are equally distributed on both new surfaces. This has been confirmed using Auger electron techniques⁶.

Earlier calculations have two major defects. First, information about the crystal structure has been based on an idealised arrangement of atoms and is therefore inaccurate. Second, electrostatic energy has either been calculated for pairs of charges on each side of the cleavage, or else a two-dimensional summation has been used.

In fact, the structure of muscovite is very different from the ideal structure. There is a considerable distortion from the ideal hexagonal arrangement of oxygen ions and the potassium ions have six coordinating oxygens rather than twelve⁷. Accurate refinements have culminated in a neutron diffraction study which located the hydrogen ions⁸.

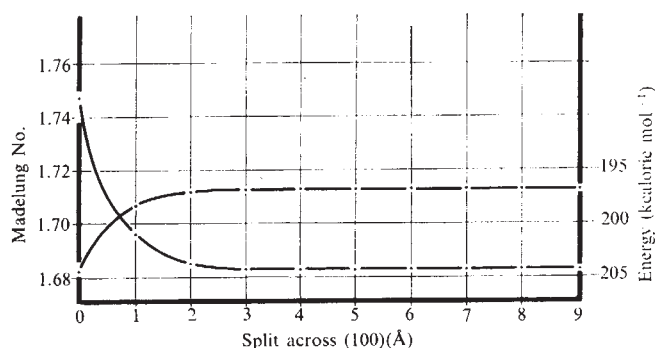


FIG. 1 A plot of the electrostatic energy (●, right axis) and Madelung number (▲, left axis) against the amount of split across the (100) face of NaCl.

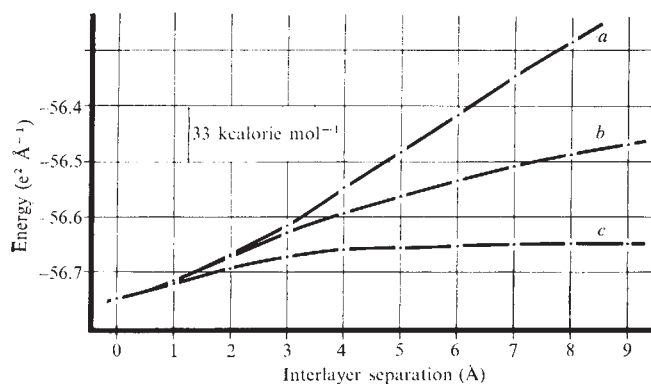


FIG. 2 A plot of electrostatic energy against the increase in the interlayer region between the aluminosilicate layers of muscovite. Curve *a* refers to the case in which the K ions remain in a plane equidistant from the two neighbouring mica layers; curve *b* is the case in which all the K ion remains on one layer and curve *c* represents an ordered arrangement with half of the K ions on each layer.

Electrostatic forces act over long distances and a correct calculation should take into account all of the atoms in the structure, and not just those on the cleavage surface. The surface energy represents the energy difference between a single crystal and one which has been separated into two parts across a plane. We have calculated the electrostatic energy of the normal crystal and of a series of structures which have an increased distance between atoms on either side of the plane. As the distance across the plane increases, the electrostatic energy approaches the energy for an infinite separation. These values give the surface energy directly.

As an example, the surface energy for the (100) face of NaCl has been calculated. The electrostatic energies of the normal and expanded structures were obtained using a computer program¹⁰. Figure 1 shows the Madelung number (left side) and the electrostatic energy (right side) against the increase in distance across the (100) face (horizontal axis). The Madelung number and electrostatic energy rapidly approach 1.68231 and $-196.87 \text{ kcalorie mol}^{-1}$ respectively, corresponding to a surface Madelung number of 0.06525 and a surface energy of $7.63 \text{ kcalorie mol}^{-1}$. These are in excellent agreement with earlier results¹¹.

The results of similar three dimensional calculations for the (001) plane of muscovite, based on the refinement of Rothbauer are shown in Fig. 2. Three distributions of interlayer potassium resulted in the three curves: curve *a* is for a structure in which the potassium ions remain in a plane equidistant from both neighboring layers; curve *b* is the case in which all of the potassium ions remain on one cleavage surface; curve *c* is for an ordered array of half of the potassium ions on each surface. Model *a* is clearly an unrealistic structure. This is evident from the energy plot which shows the greatest energy input required for cleavage. Model *b* is also energetically unfavourable but may represent the situation locally on a cleavage surface. Model *c* is the ordered array generated when the potassium ions at $z = \frac{1}{4}$ and $\frac{3}{4}$ are split so that two ions normally related by the c-face centring become crystallographically independent and remain on opposing surfaces. It is clear that for the ordered array, the electrostatic energy rapidly assumes a constant value which is the energy of an isolated mica layer. The energy difference between the normal and 'cleaved' mica is $0.098 e^2 \text{ Å}^{-1}$ ($32 \text{ kcalorie mol}^{-1}$, 891 mJ m^{-2}).

The behaviour of the three models can be understood by considering the expression for the electrostatic energy:

$$\frac{-e^2}{2} \sum_{r=1}^{\infty} \sum_{s=1}^N \frac{Z_r Z_s}{R_{rs}}$$

where e is the charge on the electron, Z is the ionic charge

in one of the atoms in the structure, N is the number of atoms in the unit cell and R is the interionic distance. The arrangement of ions in model *a* is such that as the layers separate, the K^+-K^+ interactions remain constant while the K^+-O^- interactions decrease. The result is a decrease in the electrostatic energy. Model *b* is similar but there are fewer K^+-O^- interactions which become smaller. The K^+-O^- interactions in model *c* change as in model *b*, but many of the K^+-K^+ interactions decrease.

The surface energy of muscovite in a very high vacuum (1×10^{-23} torr) is about $5,000 \text{ mJ m}^{-2}$ ($168 \text{ kcalorie mol}^{-1}$, $0.05 \text{ e}^2 \text{ \AA}^{-2}$) (ref. 3). This large value implies that the potassium ions in real crystals are not ordered, and the energy-separation curve will lie between curves *b* and *c* (Fig. 2). In addition, if there is a high rate of splitting, the chances of maintaining locally ordered regions of potassium will be less than if the rate is low. A crystal which has been rapidly split would have an experimental curve closer to *b* than would a crystal split more slowly. This is in agreement with the observation that more energy is required to cleave mica rapidly⁵. It is not sufficient to assume that an average of half of the potassium will be on each cleavage surface; the arrangement of the potassium ions on the surface is an important factor in determining the interlayer attraction.

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- ¹ Obreimoff, J. W., *Proc. R. Soc.*, **A127**, 290 (1930).
- ² Bailey, A. I., and Kay, S. M., *Proc. R. Soc.*, **A301**, 47 (1967).
- ³ Bryant, P. J., *Trans. Ninth Vacuum Symp.* (1962).
- ⁴ Bailey, A. I., and Courtney-Pratt, J. S., *Proc. R. Soc.*, **A227**, 500 (1955).
- ⁵ Deryagin, B. V., and Metsik, M. S., *Soviet Physics—Solid State*, **1**, 1393 (1959).
- ⁶ Deville, J.-P., and Goldstaub, S., *Cr. Hebd. Séanc. Acad. Sci.*, **B268**, 629 (1969).
- ⁷ Radoslovich, E. W., *Acta Crystallogr.*, **13**, 919 (1960).
- ⁸ Rothbauer, R., *Neues. Jb. Miner. Mn.*, **4**, 143 (1971).
- ⁹ Giese, R. F., *Clays and Clay Minerals*, **21**, 145 (1973).
- ¹⁰ Bauer, W., *Acta Crystallogr.*, **19**, 909 (1965).
- ¹¹ van Zeggeren, F., and Benson, G. C., *J. chem. Phys.*, **26**, 1077 (1957).

New calcium silicate phase in hydrothermally treated γ -dicalcium silicate-quartz mixtures

HYDROTHERMALLY treated γ -dicalcium silicate-quartz mixtures have been investigated by several authors as a potential calcium silicate building product which, because of its low water content, could be used as refractory or heat insulating material. The phase composition of material formed at 150–600° C and 5–300 bar, for bulk molar Ca/Si ratios between 3.0 and 1.5 has been determined by Speakman *et al.*¹. Jernejčič *et al.*^{2,3} investigated lower Ca/Si molar ratios, that is, between 1.5 and 0.5 at 180–250° C, under saturated and superheated steam conditions. Superheated steam conditions during the initial period of hydrothermal reaction gave an unknown phase with a strongly pronounced diffraction maximum of 3.15 Å in the multiphase reaction products for bulk Ca/Si ratios lower than 1.25. Using previously recorded crystallographic data for calcium silicates and calcium silicate

hydrates, the maximum of about 3.15 Å could be assigned to the gyrolite group of hydrates. These phases, however, exhibit strong basal reflections at 22 and 19 Å. These basal reflection were not observed and identification was therefore necessary, particularly because there were no signs of pseudowollastonite in samples heated to 1000° C, as should have been the case if gyrolite or truscotite or a mixture of both were originally present.

To study the silicate structure of the 3.15 Å phase, the samples were treated with 0.1 M HCl and afterwards analysed by X-ray diffraction. The patterns obtained after acid treatment showed that the 3.15 Å phase remained even when all the co-existing xonotlite had been destroyed. This observation suggests that the silicate anions in the 3.15 Å phase are relatively highly condensed. Although the samples investigated varied in their starting Ca/Si ratio and in the original phase composition, all gave the same pattern after acid treatment. It was therefore obvious that the pattern belonged to one and the same phase. Table 1 gives this pattern.

To index reflections for the 3.15 Å phase the unit cell parameters and symmetry were found by selected area electron diffraction. Microscopic observation of samples rich in the 3.15 Å phase showed lath-like oriented aggregates. Near the zero setting of the goniometer most of the particles gave a characteristic electron diffraction pattern (Fig. 1). To ensure reliable measurements of values of d^* from this pattern the 2.338 Å powder ring of aluminum was used as internal standard; this gave 11.8 and $3.7 \text{ \AA} \pm 1\%$ for the two perpendicular repeat distances. The crystals are elongated parallel to the 3.7 Å direction, which was chosen as *b*. The repeat distances are similar to the parameters *a* and *b*/2 for tobermorite, but the general features of the tobermorite (001) electron diffraction pattern are quite different from that of the 3.15 Å phase. Whereas tobermorite shows marked pseudohalving of *a* and *b*, and only those reflections which $h + k = 2n$ (for the pseudocell), the 3.15 Å phase shows a primitive (001) reciprocal lattice section. The two phases also differ in *c*. The third row spacing of the reciprocal lattice for the 3.15 Å phase was derived from series of electron diffraction patterns obtained by tilting crystals about *a*; this gave $c = 7.0 \text{ \AA}$. The electron diffraction patterns contained streaks that showed true *b* and *c* to be double those determined from the strong reflections only. The repeat distance of 7.4 Å in the *b* direction indicates that the silicate part of the structure consists of disordered dreierketten. The streaks of strong intensity, which were observed for $k + l = 2n$, indicate that the suffix $A \propto 22$ for the reciprocal lattice of the 3.15 Å phase. There are also, however, weak streaks for $k + l = 2n + 1$, so the true suffix should be $P \propto 22$ (ref 4). Electron diffraction patterns obtained by tilting

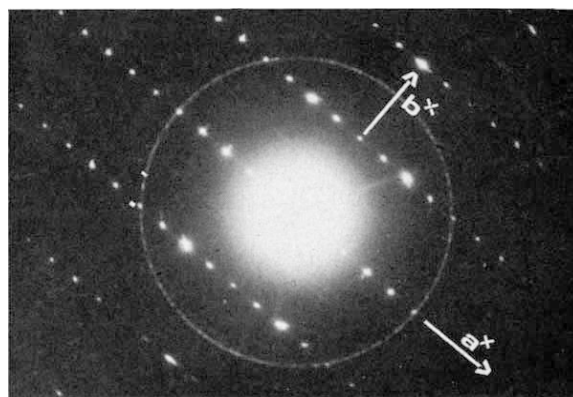


FIG. 1 Characteristic selected area electron diffraction pattern of 3.15 Å phase (corresponding to (001) reciprocal lattice section).

TABLE 1 X-ray powder data for 3.15 Å phase

d_{obs}	I/I_0	d_{cal}	h	k	l
6.99	11	7.000	0	0	2
3.971	20	3.968	3	0	0
3.660	25	3.678	3	0	2
3.230	47	3.238	1	0	4
3.210	47	3.215	1	2	2
3.150	100	3.148	2	2	0
2.805	57	2.796	2	2	2
2.710	16	2.710	3	2	0
2.610	5	2.612	3	2	2
		2.611	4	0	2
2.532	13	2.540	1	2	4
2.335	9	2.334	0	0	6
2.130	10	2.126	4	0	4
2.051	19	2.051	3	2	4
1.981	34	1.984	6	0	0
		1.982	6	0	2
1.852	32	1.855	0	4	0
		1.852	5	0	4

crystals about a^* and b^* also showed that the phase is monoclinic.

Using the unit cell parameters approximately determined from electron diffraction patterns it was possible to index all the reflections appearing on the X-ray diffraction pattern (Table 1). The unit cell parameters refined from the X-ray data are: $a = 12.02$ Å, $b = 7.42$ Å, $c = 14.14$ Å and $\beta = 98^\circ$. As already mentioned, the value of b indicates that silicate chains, similar to those existing in compounds of the wollastonite type are present in the 3.15 Å phase; consistent with this indication the 3.15 Å phase transforms into β -wollastonite at 700° C. The phase transition brought about by thermal treatment was followed on the sample richest in the 3.15 Å phase; it also contained unreacted quartz and a little xonotlite. On heating the sample to 680° C the X-ray pattern of the 3.15 Å phase did not change markedly. In a sample treated at 700° C, β -wollastonite and quartz were the only phases identified. The sample containing the highest proportion of the 3.15 Å phase was also used to evaluate approximately the Ca/Si molar ratio of the phase. Calculations using the weight fraction of unreacted quartz determined by X-ray diffraction and the loss on ignition gave a molar Ca/Si = 0.84. The ratio was also determined by electron microprobe, using a Philips EM300 electron microscope and an energy dispersive X-ray analysis system; after applying corrections⁵ an average molar ratio of 0.81 ± 0.03 was obtained. The amount of structurally important water in the 3.15 Å phase has not yet been precisely determined, but according to differential thermal analysis, thermal gravimetry and differential thermal gravimetry measurements it must be low (about 1%). Further work on the exact determination of the water content and the possible arrangement of atoms in the 3.15 Å phase is in progress.

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¹ Speakman, K., Taylor, H. F. W., Bennet, J. M., and Gard, J. A., *J. chem. Soc., A*, 1052 (1967).

² Jelenić, I., Jernejčič, J., and Kurbus, B., *Bull. Slovene chem. Soc.*, **18**, 43 (1971).

³ Jernejčič, J., Šorli, S., and Jelenić, I., *Third Yugoslav Congress on the Pure and Applied Chemistry, Ljubljana*, (1972).

⁴ Gard, J. A., *Nature*, **211**, 1078 (1966).

⁵ Walinga, J., *Internal Papers of PIT Advanced Application Group*, (1973).

New method of nucleating diamonds

SYNTHESIS of diamond from graphite in the size range <0.1 to 1 mm requires an ultra-autoclave capable of operating at about 100,000 atm and 2000° K (ref. 1). A molten metal solvent-catalyst is also needed so that the conversion may be achieved in a relatively short time (minutes). Catalysts used include chromium, manganese and tantalum plus all elements of Group VIII of the periodic table, but nickel is used most frequently. The cross hatched region of Fig. 1 shows the combinations of pressure and temperature used for diamond synthesis when nickel is the catalyst.

It is advantageous to use metal-coated diamonds in resin-bonded wheels when grinding relatively ductile materials such as cemented tungsten carbides or hard tool steels. Nickel coated grains are most commonly used for wet grinding and about 55% of the weight of diamond is deposited as nickel which corresponds to a 15% increase in volume or an increase in diameter of about 15%. The metal coating is believed not only to provide a larger area for bonding but also promote heat transfer away from the wheel surface and to retain particles in the wheel face when grains fracture due to mechanical shock or thermal fatigue.

As well as these thermal and mechanical influences the metal coating could act to decrease wear by a chemical mechanism. At atmospheric pressure diamond represents an unstable form of carbon which will tend to transform to graphite if the temperature is sufficiently high and the soft transformed graphite will be rapidly worn away. A very thin coating of nickel will smear over the surface of an active coated diamond grain and in so doing could alter the

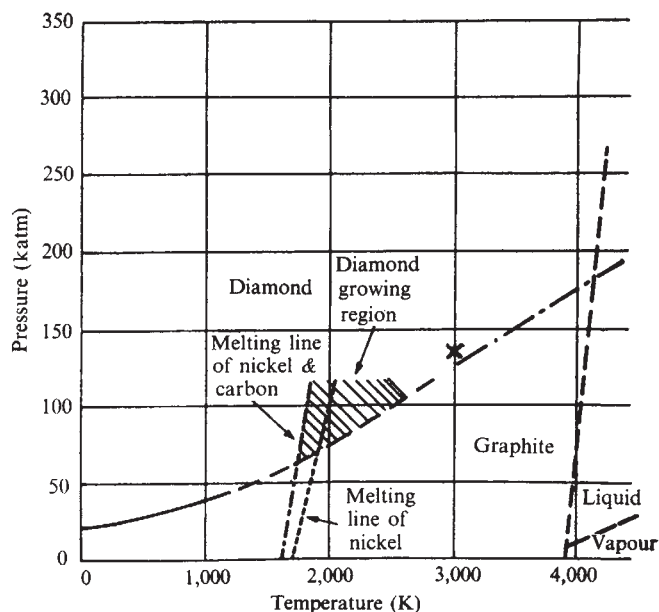


FIG. 1 Diamond-graphite equilibrium diagram with the diamond-growing region (using a nickel catalyst) shown cross hatched (ref. 2).

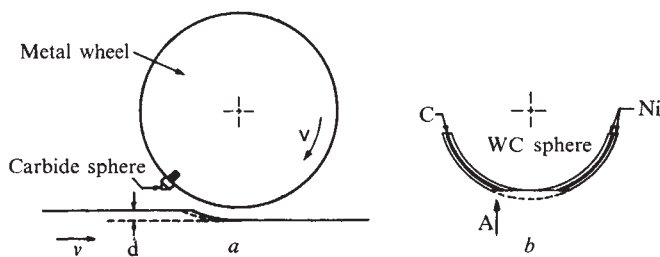


FIG. 2 a, Single-point cutting arrangement; b, tip of worn tungsten carbide sphere.

rate of wear by changing the equilibrium between the conversion of diamond to graphite and the conversion of graphite to carbon. Local conditions at the tip of the abrasive grain will be in the range of those found in the apparatus used to synthesize diamond from graphite in the presence of nickel.

When grinding steel, surface temperatures estimated as $\sim 3000^\circ\text{C}$ are obtained³ with grain-chip contact pressures as high as 2×10^6 pounds inch⁻² (130 katm)⁴. This condition marked by a cross on Fig. 1 is seen to exceed somewhat the range of operation of that used in diamond synthesis (cross hatched region). It is to be expected that under such extreme conditions the diamond nuclei produced will be extremely small, closely spaced and spontaneously formed.

The extreme conditions found at the tip of a cutting tool have been used previously to promote chemical reactions in organic synthesis in the process known as mechanical activation⁵. The very high temperatures and pressures existing at the tool tip constitute autoclave conditions without the need for an actual autoclave. This, together with the unique cleanliness of the nascent surfaces generated, causes an immediate reaction which is of great importance for organometallic reactions that are autocatalytic, such as those of the Grignard type.

To test the feasibility of generating diamond nuclei at the tip of an abrasive grain we conducted the following experiment. A tungsten carbide sphere, 1.5 mm in diameter, was coated with layers of nickel and graphite. The tungsten carbide sphere was first cleaned by sputter etching and then three thin layers were deposited in the order nickel-carbon-nickel using a sequential sputtering apparatus.

The coated tungsten carbide sphere was then cemented into a hollow head screw which in turn was threaded radially into the surface of an aluminum disk 8 inches (20 cm) in diameter. The disk was mounted on the spindle of a surface grinding machine in place of the usual grinding wheel and used to cut a groove in a piece of hardened ball bearing steel (AISI52100 steel of hardness R_c61) as shown schematically in Fig. 2. The speed of the disk (V) was in the range of conventional grinding (6,000 feet min⁻¹ or 30 m s⁻¹) while the table speed (v) and depth of cut (d) were adjusted to provide individual chips comparable in size to those in ordinary surface grinding ($v = 6$ inch min⁻¹ or 2.54 mm s⁻¹ and $d = 0.001$ inches or 25 μm). The length of groove produced was 0.5 inches (12.5 mm) which corresponds to 250 individual cuts.

After cutting, a small flat was found on the tip of the tungsten carbide sphere (Fig. 2b). When the worn surface of the composite coating was examined in a scanning electron microscope for evidence of diamond nuclei from direction A in Fig. 2a, many small nucleation sites about 1 μm in diameter were evident at the inner nickel layer (Fig. 3a). The nuclei shown in Fig. 3a are believed to be diamond nuclei generated under the extreme conditions of temperature and pressure pertaining during cutting. Figure 3b shows a similar scanning electron micrograph of the composite surface abraded by hand on fine abrasive paper (4/0 silicon carbide) at low speed. In this case the abraded specimen is subjected to relatively low temperature, and hence conditions are not

favourable for the formation of diamond. The nuclei evident in Fig. 3a are not present in this case.

We used the Debye-Scherrer X-ray diffraction technique in an attempt to verify that the nuclei evident in Fig. 3a were in fact diamond. The sample was held stationary in the camera with the X-ray beam aimed at the wear scar. Tungsten carbide, titanium carbide and nickel patterns were clearly evident. In addition, one spot (corresponding to the strongest diamond line) was also found, possibly indicating the presence of diamond. The small concentration of nuclei makes it very difficult to obtain certain verification of their structure but work is continuing on this problem.

The practical implications of the possibility of generating diamond nuclei at the tip of an abrasive grain seem to lie in at least three areas:

- (1) The rate of wear of diamond grinding wheels could be reduced;
- (2) A source of diamond seeds for epitaxial growth of diamond by vapour phase deposition of diamond would be opened up;
- (3) It would provide a convenient method of studying the formation of new high temperature, high pressure stable phases of materials.

Some of the graphite formed by degradation of diamond during wear may be reconverted to diamond if nickel is present. The presence of nickel would thus alter the net rate of conversion of diamond to graphite. If so, it should be advantageous to provide a source of graphite to the diamond grain tip in addition to that coming from the conversion of diamond.

A diamond phonograph stylus might be made by coating an aluminum oxide cone of proper geometry with alternate layers of nickel and graphite. By making a few cuts in each of several directions seeds would be generated on the surface which could then be enlarged and subsequently grown together by epitaxial growth. The result would be a relatively large item of inexpensive material coated with a thin layer of diamond in the critical region only.

Although there is some evidence for the nucleation of diamonds by the technique reported here, it is not completely conclusive. But since the method is novel, simple and inexpensive, it could lead to many new important applications similar to those presented here. More important, if this

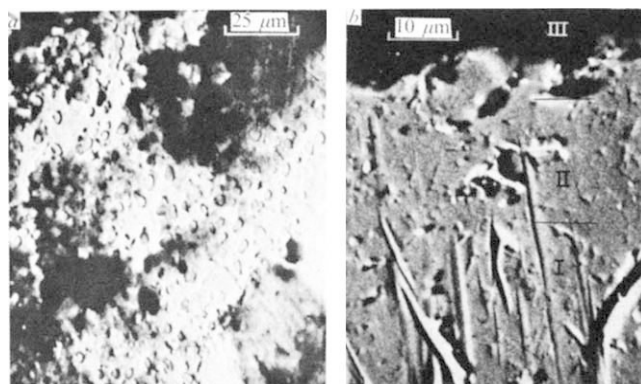


FIG. 3 a, Scanning electron micrograph of portion of inner nickel layer showing many (possibly diamond) nuclei; b, scanning electron micrograph of inner nickel layer abraded by hand at low speed. No nuclei seem to be present in this case. I, Tungsten carbide sphere; II, inner nickel layer; III, carbon layer.

mechanism is operating in grinding with diamond abrasive, it sheds light on the basic wear mechanism and suggests means for reducing the rate of wear in practice.

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- ¹ Bundy, F. P., Hall, H. T., Strong, H. M., and Wentorf, R. H., *Nature*, **176**, 51 (1955).
² Bovenkerk, H. P., Bundy, F. P., Hall, H. T., Strong, H. M., and Wentorf, R. H., *Nature*, **184**, 1094 (1959).
³ Mayer, J. E., and Shaw, M. C., *Lubric. Engng*, **13**, 21 (1957).
⁴ Marshall, E. R., and Shaw, M. C., *Trans. ASME*, **74**, 51 (1952).
⁵ Shaw, M. C., *Trans. ASME*, **70**, A-137 (1948).

Late Pleistocene desiccation along the White Nile

MORE than a century ago Baker¹ described the Blue Nile as a mountain stream, rising and falling with great rapidity, and the White Nile as a river of lake origin, flowing through vast treeless swamps in a land of "malaria, marshes, mosquitoes, misery". Three decades later Hull² noted that the flow of the main Nile had once been greater, and early this century a controversy arose which persists to this day. Was Nile discharge greater during the glacial periods of Europe and East Africa? Or were the glaciations coeval with tropical aridity?

Hume and Craig³ maintained that glacial ice caps in East Africa would displace the monsoon winds southwards, reducing precipitation over Ethiopia and thereby curtailing the flow of the Sobat, Blue Nile and Atbara, which currently account for more than 90% of the main Nile flood. Penck⁴ considered that when the northern Sahara was wet the southern Sahara was dry and encroached farther south. During a glacial period the arid belt lay 5° nearer the equator as a result of lower global temperatures and weakened atmospheric circulation.

More recently Fairbridge^{5,6} has equated Nile aggradation with diminished discharge during glacials, arguing that, since the Nile is now well able to transport its load to the delta, widespread silt deposition above the first cataract must imply reduced competence. Theoretical reconstructions of full glacial climates^{7,8} posit drier climates in areas as far afield as the south-west United States⁹, south-east Australia¹⁰, and parts of the Mediterranean littoral¹¹. In essence, lower temperatures imply lower evaporation rates⁷, which should result in lower rainfall^{5,7}.

Since 1960 many workers have reported evidence which seems to oppose the glacial aridity model and to favour the view that Nile discharge was greater than now during much of the late Pleistocene. Butzer and Hansen¹² referred to "revolutionary changes" in the late Pleistocene hydrographic regime of the Nile, and concluded that the wetter phases were synchronous in both Ethiopia and Egypt, except during deposition of the Masmas Formation at Kom Ombo about 20,000 yr BP. De Heinzelin^{13,14} also equated wadi activity in Nubia with higher rainfall in Ethiopia, Nile aggradation coinciding with conditions of maximum flow. Wendorf and Said¹⁵ described a phase of rising Nile level and widespread silt deposition in Nubia from 20,000 to 12,000 yr BP; and Wendorf and colleagues¹⁶ have distinguished three aggradational phases in Nubia between 16,000 and 12,000 yr BP, which were coeval with aridity in that region.

Three contradictory palaeoclimatic models purport to explain late Pleistocene aggradation by the main Nile. One equates aggradation with reduced Nile discharge and glacial aridity in Ethiopia^{5,6}. The second equates aridity with river incision, and greater Nile discharge with aggradation and wetter conditions in Nubia^{12,13}. The third equates siltation with high Nile levels and local aridity¹⁶. To resolve these issues the White Nile and the Blue Nile require separate study. We now present evidence which suggests that the White Nile had a much reduced and highly seasonal flow during the late Pleistocene before it rose to several metres above its modern unregulated flood level between 12,000 and 8,000 yr BP.

During a survey of prehistoric sites along the White Nile in January-March 1973 in association with J. D. Clark and an archaeological team from Berkeley, we collected microcrystalline dolomite and calcite from beneath subfossil freshwater shells which were laid down between 11,000 and 11,500 yr BP. When the shells were deposited, the White Nile flood level was 378 m, which is 5 m above the mean minimum and 2.5 m above the mean maximum water level¹⁹ at Esh Shawal before the Jebel Aulia dam was built (Fig. 1). The carbonate came from a depth of 3.75–3.90 m at site 2B and of 4.0–4.8 m at site 2. Above the carbonate was a layer of grey to yellow fluviatile sand from 0.5 to 1.1 m thick, locally with a basal admixture of clay and coarse quartz sand (Fig. 2).

An almost horizontal layer of greyish green lacustrine clay capped the sand and extended about 13 km west of the present White Nile to near Jebel et Tomat in latitude 13°36'N. The green clay was up to 1.5 m thick, with a surface elevation of 377.8 m in the west rising imperceptibly to 379.5 m some 7 km further east. Immediately above the green clay was a discontinuous shell-bed generally 5–10 cm thick made up of unbroken freshwater shells of *Cleopatra bulimoides* (Oliv.), *Melanoides tuberculata* (Müller) cf.

TABLE 1 Carbon-14 ages of shells in sediments bordering the White Nile

Site	Sample	Depth (m)	Elevation of base of shell beds (m)	Location	Age (yr BP)	Remarks
1	I 1486	1.45–1.70	377.3	13°35'N 32°41'E	11,300 ± 400	High river level ¹⁷
2	SUA-75	1.2–1.4	377.3	13°36'N 32°40'E	11,250 ± 220	High river level
3	I 1485	1.8–1.9	376.5	13°56'N 32°22'E	8,370 ± 350	High river level ¹⁷
3	SUA-68	1.2–1.4	377.0	13°56'N 32°32'E	8,130 ± 225	High river level
4	SUA-72	0.3–0.45	~398	15°23'N 32°22'E	8,400 ± 150	Local lakes full
4	SUA-74	0.45–0.60	~398	15°23'N 32°22'E	7,620 ± 130	Local lakes full
5	SUA-71	0.08–0.12	~401	15°22'N 32°21'E	7,870 ± 140	Local lakes full
6	SUA-73	0.12–0.20	~401	15°22'N 32°21'E	6,990 ± 90	Local lakes full
7	SUA-211	0–0.35	~378.5	13°34'N 32°34'E	5,500 ± 100	Pila midden on point-bar
8	SUA-67	0.4–0.8	384.2	13°36'N 32°44'E	4,540 ± 200	Top of dark cracking-clay
9	SUA-70	0.5–0.65	~377	13°56'N 32°21'E	3,030 ± 80	Pila midden on point-bar
10	SUA-212	Living		White Nile	150% modern	Living <i>Corbicula</i> shell
11	SUA-215B	Modern		White Nile	152.6% modern	Modern <i>Pila wernei</i> shell

Unless specified, all dates are from the Radiocarbon Laboratory, University of Sydney.

dautzenbergi Pilsbry and Bequaert, *Corbicula africana* (Krauss) and *Biomphalaria sudanica* (Martens). At site 2, fish spines and vertebrae were mixed among the shells, which gave a ^{14}C age of $11,350 \pm 220$ yr BP, in close agreement with the age of $11,300 \pm 400$ obtained from site 1^{17,20} (Table 1). Above the shell bed was a dark, cracking clay up to a metre or more thick, which accumulated in swamps left by the receding White Nile. Between about 12,000 and 8,000 yr BP, the White Nile remained high and formed an extensive lake up to 30 km or more wide (site 3, Table 1). Small lakes also existed west of the White Nile some 20 km north-west of Jebel Aulia (sites 4 to 6) between about 8,500 and 7,000 yr BP²¹, after which they remained quite dry.

Geochemical and palynological evidence indicate that between >14,500 and 12,000 yr BP Lake Victoria had no outlet²², the regional climate was dry, and dry forest grew on Ruwenzori²³. The White Nile was deprived of water from the Uganda lakes, so that its flow dwindled to a mere seasonal trickle. Dune formation was active along both banks of the White Nile between Kosti and El Geteina^{24,25} (Fig. 1) and deposits of very pure carbonate accumulated in or along the river, which contained abundant calcium and magnesium, indicative of the high salinity concentrations brought about by increasing evaporation downstream—a feature of modern rivers in arid areas²⁶. Site 2B contained both dolomite and calcite crystals, suggesting that when the dolomite was form-

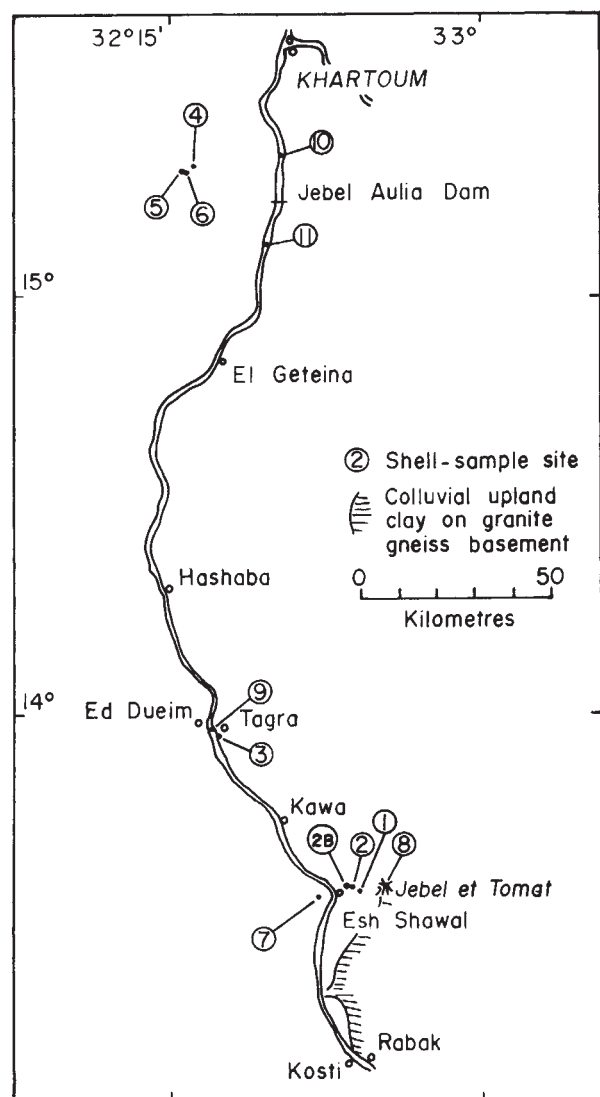


FIG. 1 Location of dated shell beds along the White Nile.

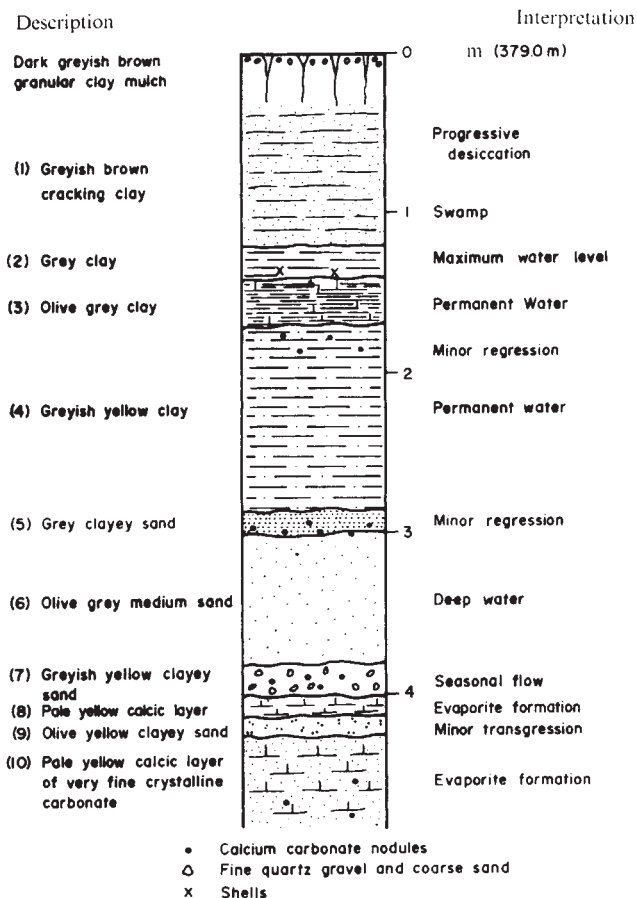


FIG. 2 Esh Shawal, section 2B.

ing magnesium-rich calcite was already abundant, and the ratio of dissolved Mg to Ca was at least 7 (ref. 27). It is worth noting that the levels of Lake Chad²⁸, Lake Rudoli²⁹ and Lake Nakuru³⁰ were also very low at this time, and dunes were active in now vegetated parts of Nigeria³¹.

About 12,000 yr BP Lake Victoria overflowed, the level of the White Nile rose, and the dunes between Kosti and Ed Dueim became partly buried by alluvial clay. After 8,000 yr BP the level of the White Nile fell, dark clays accumulated in the swamps bordering the river until about 4,000 yr BP (Table 1, site 8), after which Neolithic man and increasing climatic desiccation accelerated the trend towards semi-desert conditions. The fixed dunes of Kordofan and the even flow of the modern White Nile indicate that the late Pleistocene dry period was more arid than the present semi-arid climate of central Sudan, and it is possible that the White Nile may have ceased to flow into the main Nile, which would thus have been even more seasonal in its regime than now.

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- ¹ Baker, Sir Samuel, *The Albert N'Yanza. Great Basin of the Nile and Explorations of the Nile Sources*, 1, 5-6, 54 (Sidgwick and Jackson, London, 1866; reprinted 1962).
- ² Hull, E. Q., *J. geol. Soc. Lond.*, **52**, 308 (1896).
- ³ Hume, W. F., and Craig, J. I., *Rep. Br. Ass. Advnt. Sci.*, **81**, 382 (1911).
- ⁴ Penck, A., *Scott. geogr. Mag.*, **30**, 281 (1914).
- ⁵ Fairbridge, R. W., *Nature*, **196**, 108 (1962).
- ⁶ Fairbridge, R. W., *Kush*, **11**, 96 (1963).
- ⁷ Flohn, H., *Erdkunde*, **7**, 266 (1953).
- ⁸ Barry, R. G., and Williams, J., *9th INQUA Congress, Christchurch, Abstracts*, 12 (1973).
- ⁹ Galloway, R. W., *Ann. Ass. Am. Geogr.*, **60**, 245 (1970).
- ¹⁰ Galloway, R. W., *J. Geol.*, **73**, 603 (1965).
- ¹¹ Butzer, K. W., *Environment and Archeology* (second ed.), 302, 311 (Methuen, London, 1971).
- ¹² Butzer, K. W., and Hansen, C. L., *Desert and River in Nubia* (University of Wisconsin Press, 1968).
- ¹³ Heinzelin, J. de, in *Background to Evolution in Africa*, 313 (edit. by Bishop, W. W., and Clark, J. D.), (University of Chicago Press, 1967).
- ¹⁴ Heinzelin, J. de, in *The Prehistory of Nubia*, 1, 19 (edit. by Wendorf, F.), (Southern Methodist University Press, Texas, 1968).
- ¹⁵ Wendorf, F., and Said, R., *Nature*, **215**, 244 (1967).
- ¹⁶ Wendorf, F., Said, R., and Schild, R., *Science*, **169**, 1161 (1970).
- ¹⁷ Williams, M. A. J., *Nature*, **211**, 270 (1966).
- ¹⁸ Williams, M. A. J., and Adamson, D. A., *Geogr. J.* (in the press).
- ¹⁹ Hunting Technical Services, *Roseires Soil Survey, Report No. 6* (1964).
- ²⁰ Whiteman, A. J., *The Geology of the Sudan Republic*, 114 (Oxford University Press, 1971).
- ²¹ Williams, M. A. J., Medani, A. H., Talent, J. A., and Mawson, R., *Sudan Notes and Records* (in the press).
- ²² Kendall, R. L., *Ecol. Monogr.*, **39**, 121 (1969).
- ²³ Livingstone, D. A., *Ecol. Monogr.*, **37**, 25 (1967).
- ²⁴ Warren, A., *Z. geomorph. Suppl.*, **10**, 154 (1970).
- ²⁵ Williams, M. A. J., *J. Soil Sci.*, **19**, 367 (1968).
- ²⁶ Gibbs, R. S., *Science*, **170**, 1088 (1970).
- ²⁷ Müller, G., Irion, G., and Förstner, U., *Naturwissenschaften*, **59**, 158 (1972).
- ²⁸ Servant, M., *Séquences Continentales et Variations Climatiques: Évolution du Bassin du Tchad au Cénozoïque Supérieur*. (ORSTOM, Paris, 1973).
- ²⁹ Butzer, K. W., Brown, F. H., and Thurber, D. L., *Quaternaria*, **11**, 15 (1969).
- ³⁰ Butzer, K. W., Isaac, G. L., Richardson, J. L., and Washbourn-Kamau, C., *Science*, **175**, 1069 (1972).
- ³¹ Burke, K., Durotoye, B., and Whiteman, A. J., *W. Afr. J. Archaeol.*, **1**, 1 (1971).

BIOLOGICAL SCIENCES

Non-specific 'pairing' of DNA molecules by recombination enzyme of *Bacillus subtilis*

ATP-dependent deoxyribonuclease(s) (ATP-DNase) is involved in the genetic recombination process in *Escherichia coli*¹⁻³, *Diplococcus pneumoniae*⁴ and *Bacillus subtilis*⁵ and may also play an important part in DNA repair⁶⁻⁸, DNA replication⁹ and cell growth¹⁰. ATP-dependent or ATP-stimulated DNases have also been isolated and characterised in other microorganisms¹¹⁻¹⁴. We reported the purification and properties of this enzyme in *B. subtilis*^{15,16} and noted that at the 600-fold purification level, the enzyme requires ATP for the hydrolysis of double stranded (ds) DNA, but not for single stranded (ss) DNA¹⁶. Since the activities on ds and ssDNA appear to reside in the same enzyme, we suggested the possibility that ATP consumption is somehow linked to the active unwinding of dsDNA before the DNase can hydrolyse phosphodiester bonds¹⁶. Recently, Friedman and Smith²⁴ have also proposed an active role of ATP in dsDNA unwinding by *Haemophilus influenzae* enzyme, which is based on the structural feature of intermediate product DNA. To examine this possibility further, we tried to visual-

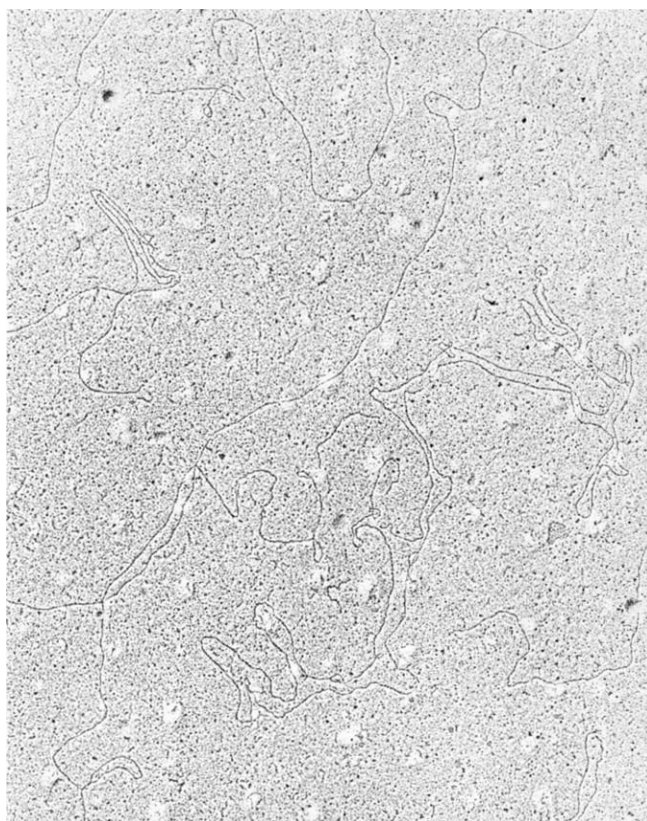


FIG. 1 Electron microscopic study of ATP-DNase-DNA complex: control. The reaction mixture (50 μ l) with 0.5 mM ATP was incubated at 0° C for 2 min and at 32° C for 3 min without enzyme. $\times 37,254$.

ize the unwound portion of DNA and the DNA-enzyme complex by electron microscopy. Although the visual evidence is still ambiguous, an unexpected observation has been made. We report here the unusual property of *B. subtilis* ATP-DNase which apparently brings DNA molecules into close proximity or causes non-specific 'pairing' of dsDNA molecules.

ATP-dependent DNase was purified from *B. subtilis* sonicate as reported previously¹⁶. The sonicate of cells was centrifuged at 40,000g for 20 min. The supernatant was further centrifuged at 100,000g for 90 min, followed by streptomycin sulphate and ammonium sulphate fractionations. The fraction between 40 and 60% saturation of ammonium sulphate was further fractionated by agarose column (A 5m, Bio-Rad Laboratories) and DEAE-cellulose column chromatography. The DEAE-cellulose purified enzyme (600-fold purified) will be called here Fraction VI enzyme in conformity with the previous paper¹⁶. This enzyme was purified

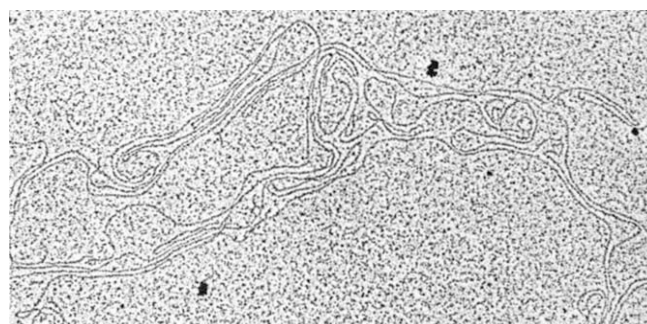


FIG. 2 Electron microscopic study of ATP-DNase-DNA complex. The reaction mixture (50 μ l) with ATP (0.5 mM) and one unit of Fraction VI enzyme (5.2 μ g protein). Incubation was for 2 min at 32° C. $\times 52,500$.

by glycerol gradient centrifugation to a final purity of approximately 5,000-fold. This latter enzyme preparation is called Fraction VIII.

The activity of ATP-DNase was measured by the release of acid-soluble DNA fragments from tritiated *B. subtilis* DNA. The DNA was phenol-purified according to the procedure of Saito and Miura¹⁷. Unless otherwise noted, the 'standard reaction mixture' (0.5 ml) contained the following: 25 mM Tris-maleate buffer (pH 7.5), 7 mM MgCl₂, 6 mM 2-mercaptoethanol, 0.5 mM ATP, 30 μ M nucleotide equivalent tritiated DNA, and 1–5 units of enzyme. One unit of enzyme activity hydrolyses 1 nmol of DNA nucleotide during 30 min incubation at 32° C.

The electron microscopic study of ATP-DNase on dsDNA was carried out as follows. Unless otherwise indicated, reaction mixtures contained the same components as the standard reaction mixture in the same concentrations except that the total volume was reduced to 50 μ l and 0.14 μ g of non-labelled phage λ DNA was used. Routinely a reaction was terminated by adding glutaraldehyde to the concentration of 10 mM at 0° C. The sample was kept for at least 10 min at 0° C and an aliquot was diluted with 0.01 M Tris-HCl, 0.001 M EDTA buffer, pH 9.0, to give 0.5 to 1 μ g ml⁻¹ DNA. Formamide and cytochrome *c* were added to final concentrations of 50% (v/v) and 0.01%, respectively. The sample was mixed thoroughly, and a few drops were spread on a water surface. DNA was picked up on carbon coated formvar grids. The sample was stained for 30 s in 0.001% solution of uranium chloride in ethanol, washed briefly in 100% ethanol and air dried. Samples were shadow-casted with 80% Pt:20% Pd in a Denton DV52 vacuum evaporator and observed in a Phillips 300 electron microscope.

Phage λ DNA molecules incubated without enzyme in the presence of ATP and glutaraldehyde (control) were well spread and the majority of the molecules could be traced from

end to end (Fig. 1). The control in the absence of ATP and glutaraldehyde showed similar pictures. When ATP-DNase of Fraction VI was present in the reaction mixture, however,

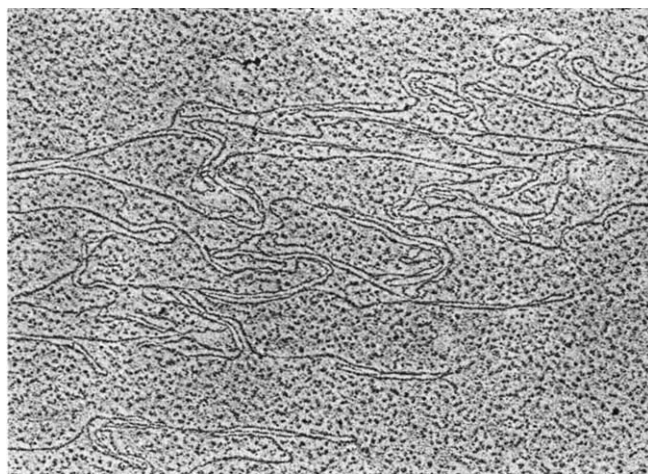


FIG. 4 Electron microscopic study of ATP-DNase-DNA complex. The reaction mixture (50 μ l) with ATP (0.5 mM) and one unit of Fraction VIII enzyme (0.7 μ g protein). Samples with one unit of enzyme purified on a glycerol gradient (Fraction VIII) were prepared and examined according to the procedure described in the text. $\times 64,220$.

a large portion of each DNA molecule was closely lined or 'paired' (non-sequence-specific pairing) (Fig. 2). Similar 'pairing' was also observed when ATP was withheld from the reaction mixture. Thus, omission of ATP from the reaction mixture in the presence of the enzyme did not significantly change the degree of 'pairing'. The degree of non-specific pairing was clearly dependent on the enzyme concentration in the reaction mixture (compare Figs. 2 and 3). In separate experiments, we have examined samples incubated with serially diluted enzyme (Fractions VI and VIII) in the electron microscope. The results showed that as the concentration of enzyme was reduced, the degree of 'pairing' also decreased. To try to eliminate the possibility that the non-specific pairing of DNA observed may be caused by some other contaminating proteins in the preparation, similar experiments to those described above using 600-fold purified enzyme (Fraction VI) were carried out using the same number of activity units of the 5000-fold purified enzyme (Fraction VIII). Figure 4 shows essentially the same degree of 'pairing' as that obtained using Fraction VI. In this case, also, omission of ATP gave a similar degree of 'pairing'.

Since the molecular weight of ATP-DNase of *B. subtilis* is estimated by sedimentation to be approximately 260,000, and since the enzyme purified on the glycerol gradient is fairly homogeneous (Ohi, Donovan and Sueoka, unpublished data), the reaction mixture of Fig. 4 contains approximately 1.5×10^{12} enzyme molecules and 2.5×10^9 molecules of λ DNA. If we assume that all enzyme molecules bind to DNA under this condition and also bind at random (for example, in a non-cooperative fashion) the average distance between enzymes would be 80 base pairs.

Other proteins, however, might cause similar non-specific pairing of DNA molecules as a result of cross-linking caused by glutaraldehyde fixation. Under our experimental conditions, addition of lysozyme to the reaction mixture in concentrations up to 500 μ g ml⁻¹ in place of ATP-DNase did not promote the non-specific pairing of phage λ DNA. In this respect, it should be noted that the reaction mixture contained 104 μ g ml⁻¹ (Fig. 2) and 520 μ g ml⁻¹ (Fig. 3) protein with Fraction VI enzyme and 14 μ g ml⁻¹ protein with Fraction VIII. Furthermore, various proteins have been reported not to cause such an effect on DNA: T4 gene 32 protein,



FIG. 3 Electron microscopic study of ATP-DNase-DNA complex. The reaction mixture (50 μ l) with ATP (0.5 mM) and 5 units of Fraction VI enzyme. Incubation was as in Fig. 2. $\times 60,000$.

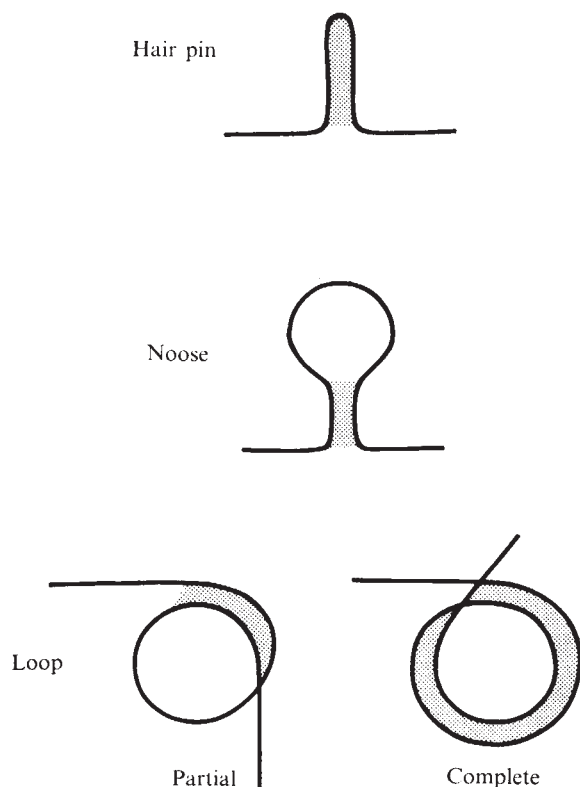


FIG. 5 Three basic configurations of dsDNA to dsDNA 'pairing'. The solid line represents double-helical DNA and the shaded area indicates pairing. The various configurations in Fig. 3 can be explained by combinations of these basic configurations.

RNA polymerase²⁰, cytochrome *c*, trypsin, chymotrypsin, dipropylfluorophosphate-treated trypsin and chymotrypsin, RNase, methylated-albumin, and lysozyme²¹.

Winder and Sastry²² reported that ATP-DNase of *Micrococcus smegmatis* formed a long-lived enzyme-DNA complex which could be trapped on the Millipore filter and assayed for its activity. Shemyakin *et al.*²³ also demonstrated the formation of the *B. subtilis* enzyme-DNA complex by glycerol gradient centrifugation. In separate experiments we have confirmed the existence of the enzyme-DNA complex by the Millipore filter method. These observations indicate that the ATP-DNase can form a stable complex with DNA. There are two possible modes of binding of the enzyme to DNA: one which enables the DNase to work in unwinding and hydrolysis, and one which causes the non-specific 'pairing' of DNA molecules.

From the present study, the detailed structure of the 'pairing' cannot be specified. The distance between the paired dsDNAs cannot be defined. DNA in Fig. 3 (high enzyme concentration) shows tight pairing, while DNA in other figures with 1/5 enzyme shows rather loose pairing. The difference may come from a higher resistance in the former preparation to stretching during the spreading with cytochrome *c*.

There has been no evidence of strand opening in any of the preparations we have looked at so far. This is not surprising since any strand separation should quickly be followed by annealing. All features of pairing in Fig. 3 can be accounted for by the three basic configurations schematically shown in Fig. 5 without introducing strand opening of double-stranded DNA. It is also clear that the 'pairing' is not limited to two dsDNAs. The sticking of more than two dsDNAs is seen in Fig. 3.

In conclusion, we have found that the ATP-DNase of *B. subtilis* brings DNA molecules into close proximity. We do not know at present the functional meaning of this 'pairing' nor its physicochemical basis. Since the enzyme is involved

in genetic recombination, however this property of the enzyme may have some significance in the mechanism of recombination and some other types of DNA-DNA interactions *in vivo*.

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¹ Buttin, G., and Wright, M., *Cold Spring Harbor Symp. quant. Biol.*, **33**, 259 (1968).

² Oishi, M., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 1292 (1969).

³ Barbour, S. D., and Clark, A. J., *Proc. natn. Acad. Sci. U.S.A.*, **65**, 955 (1970).

⁴ Vovis, G. F., and Buttin, G., *Biochim. biophys. Acta*, **224**, 42 (1970).

⁵ Chestukhin, A. V., Shemyakin, M. F., Kalinina, N. A., and Prozorov, A. A., *FEBS Lett.*, **24**, 121 (1972).

⁶ Clark, A. J., *A. Rev. Microbiol.*, **25**, 437 (1971).

⁷ Cooper, P. K., and Hanawalt, P. C., *J. molec. Biol.*, **67**, 1 (1972).

⁸ Masker, W. E., and Hanawalt, P. C., *Proc. natn. Acad. Sci.*, **70**, 129 (1973).

⁹ Brazill, G. W., Hall, R., and Gross, J. D., *Nature new Biol.*, **233**, 281 (1971).

¹⁰ Haefner, K., *J. Bact.*, **96**, 652 (1968).

¹¹ Anai, M., Hirahashi, T., and Takagi, Y., *J. biol. Chem.*, **245**, 767 (1970).

¹² Friedman, E. A., and Smith, H. O., *J. biol. Chem.*, **247**, 2346 (1972).

¹³ Winder, F. G., and Lavin, M. F., *Biochim. biophys. Acta*, **247**, 542 (1971).

¹⁴ Anai, M., *Seikagaku*, **39**, 167 (1967).

¹⁵ Sueoka, N., Matsushita, T., Ohi, S., O'Sullivan, A., and White, K., in *DNA Synthesis in Vitro*, 386-404 (University Park Press, Baltimore).

¹⁶ Ohi, S., and Sueoka, N., *J. biol. Chem.*, **248**, 7336 (1973).

¹⁷ Saito, H., and Miura, K., *Biochim. biophys. Acta*, **72**, 619 (1963).

¹⁸ Delius, H., Mantell, N. J., and Alberts, B., *J. molec. Biol.*, **67**, 341 (1972).

¹⁹ Sigal, N., Delius, H., Kornberg, T., Gefter, M. L., and Alberts, B., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3537 (1972).

²⁰ Bick, M. D., Lee, C. S., and Thomas, C. A., jun., *J. molec. Biol.*, **71**, 1 (1972).

²¹ Kleinschmidt, A. K., in *Methods in Enzymology*, Vol. **XII**, part B, *Nucleic Acids*, 361 (1968).

²² Winder, F. G., and Sastry, P. A., *FEBS Lett.*, **17**, 27 (1971).

²³ Shemyakin, M. F., Chestukhin, A. V., Kalinina, N. A., and Prozorov, A. A., *FEBS Lett.*, **31**, 31 (1973).

²⁴ Friedman, E. A., and Smith, H. O., *Nature new Biol.*, **241**, 54 (1973).

Zinc ions inhibit replication of rhinoviruses

THE rhinoviruses comprise a subgroup of the picornaviruses, distinguished by their acid lability. They are medically important as the infectious agents of the common cold in man. Although there have been reports of chemical agents which inhibit rhinovirus replication, in only a few instances has the mode of antiviral action been demonstrated at a biochemical level^{1,2}.

Although metal ions are well known cofactors in many biological processes, and are inhibitory to some enzymatic reactions^{3,4}, they have received scant attention in virology. We have now examined the abilities of the following metal ions to affect the replication of twelve different picornaviruses in HeLa cells: cadmium, calcium, cobalt, copper, magnesium, manganese, mercury, molybdenum, nickel and zinc. They were supplied as the chloride or acetate, and were tested at 1, 0.1 and 0.01 mM. We used an agar overlay assay described before⁵, but omitted DEAE-dextran. The metals were in-

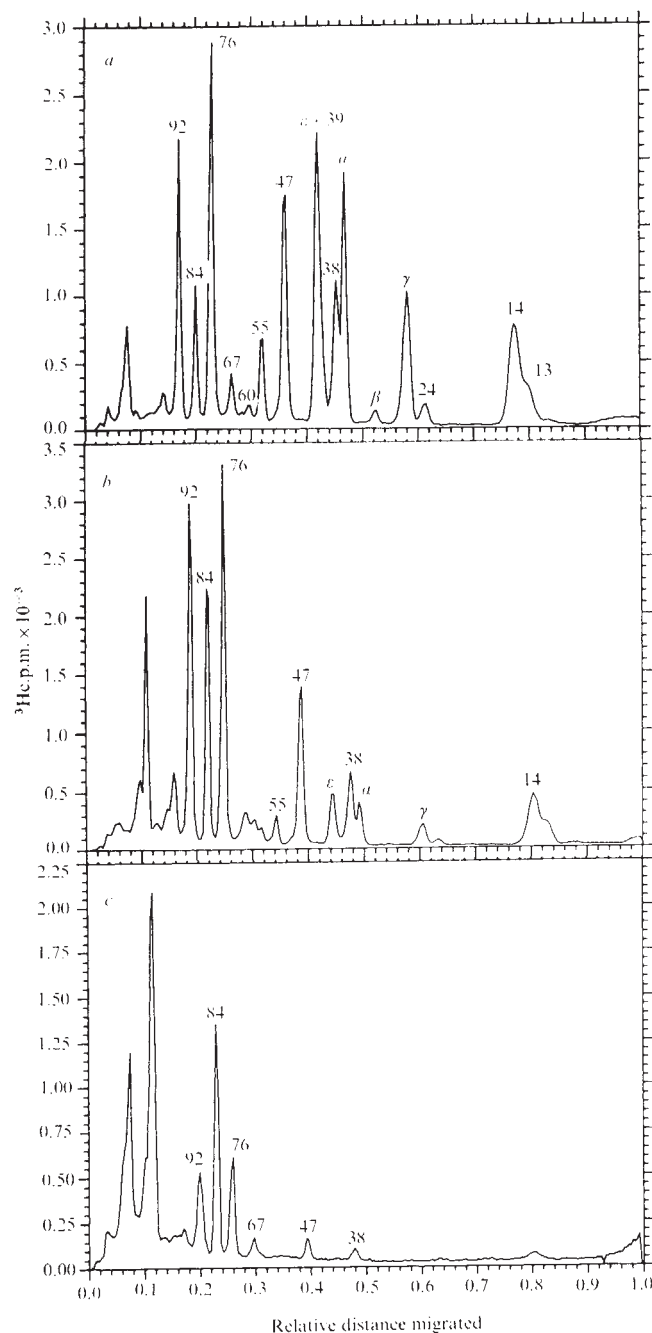


FIG. 1 Inhibition of cleavages of HRV-1A polypeptides by zinc. A suspension culture of HeLa cells (4×10^6 cells ml^{-1}) was infected with 100 PFU per cell of HRV-1A in the presence of $5 \mu\text{g ml}^{-1}$ of actinomycin D at 34°C . By 4 h after infection host protein synthesis was almost completely inhibited. Identical samples were labelled 4–5 h after infection with $50 \mu\text{Ci ml}^{-1}$ of a ^3H -amino acid mixture in the absence of added zinc (a), in the presence of 0.1 mM ZnCl_2 (b), and in the presence of 0.8 mM ZnCl_2 (c). The whole cells were then solubilised with 1% sodium dodecyl sulphate (SDS), dialysed, and subjected to electrophoresis on $0.6 \times 27 \text{ cm}$ SDS-polyacrylamide gels⁸. Migration was from left to right. The gels were then fractionated and radioactivity was measured with a liquid scintillation spectrometer. The data were analysed using a PDP-10 computer (Digital Equipment Corp.) and the electropherograms were plotted using a CalComp plotter. The gel patterns are photographs of the actual computer output.

incorporated into the agar and added to the HeLa cell monolayers after the virus had been attached to the cells for 1 h. Activity of a particular metal on a virus type was scored as the percentage reduction of the number of virus plaques in the presence of the metal compared with untreated cell

cultures. Toxicity to the cells was not measured quantitatively, but estimated by the loss of stained cells from the plastic Petri dishes after 4 d of incubation in the presence of a given concentration of the metal. If the cells displayed a toxic response, the antiviral activity of the metal at that concentration was not counted.

Only zinc displayed antiviral activity in non-toxic concentrations. Table 1 shows that eight out of nine rhinoviruses were sensitive to 0.1 mM zinc. There was a spectrum of sensitivities, with type 1A being most and type 14 least inhibited. One human rhinovirus, type 5, and two types of poliovirus were insensitive.

To determine the kinetics of zinc inhibition, HeLa cell monolayers infected with HRV-1A were exposed to 0.1 mM zinc at various times during a single replication cycle and the final yield of virus was determined in each case. The results indicated that the addition of zinc at any time during viral replication immediately inhibited further formation of infectious virions.

Two easily assayed processes of picornavirus replication are RNA and protein synthesis. Measurements of the effect of 0.1 mM zinc on total incorporation of ^3H -uridine, or amino acids into HRV-1A macromolecules, indicated that viral RNA and protein synthesis were reduced only by about 10–20%. This slight reduction was not sufficient to explain the marked inhibitions seen in plaque tests (Table 1).

The viral RNA acts as a message in the infected cell to direct the synthesis of the viral polypeptides. Some of the large polypeptides generated during translation are precursor molecules which undergo proteolytic cleavages to produce the smaller viral polypeptides^{6–9}. The most dramatic effect of zinc was to inhibit some of these cleavages of the HRV-1A polypeptides. Figure 1a shows the normal pattern of labelled viral polypeptides formed during 1 h of labelling. Addition of 0.1 mM zinc with the radioactive amino acids prevents post-translational cleavages and causes the accumulation of a set of large precursor polypeptides (Fig. 1b). We found that different cleavages were sensitive to different concentrations of zinc, and that progressively larger polypeptides could be accumulated by increasing the zinc concentration (Fig. 1b and c). Zinc also prevented the cleavage of HRV-2 precursor polypeptides in HeLa cells.

It is significant that the two types of poliovirus which could produce plaques in the presence of 0.1 mM zinc (Table 1) demonstrated normal intracellular polypeptide patterns (not shown) at a zinc concentration which markedly blocked cleavage of HRV-1A polypeptides. In suspension cultures and at higher concentrations of zinc, however, there is marked inhibition of cleavage of certain polio- and EMC viral polypeptides (B.E.B. and B.D.K., in preparation). The cleavage reaction most sensitive to zinc inhibition is the conversion of the capsid precursor, polypeptide 92, (Fig. 1b) to ϵ , γ , and α , the HRV-1A capsid polypeptides^{6–9}. Although other explanations are possible, we suggest that zinc interacts with the HRV-1A capsid proteins (B.D.K. and B.E.B., in

TABLE 1 Plaque-forming ability of picornaviruses on HeLa cells in the presence of 0.1 mM zinc chloride

Virus	% Reduction in No. of plaques
Human rhinovirus (HRV) 1A	99.99
HRV-1B	99.9
HRV-2	97
HRV-3	99
HRV-5	<10
HRV-14	85
HRV-15	99
HRV-39	99
HRV-51	90
Equine rhinovirus (Plummer strain)	95
Poliovirus type 1 (Mahoney)	0
Poliovirus type 2 (P712ch2ab)	0

preparation), blocking conversion of the precursor and rapidly preventing maturation of viral RNA and capsid polypeptides.

This report represents a direct demonstration that a chemical which blocks cleavage of viral protein precursors can act (in tissue culture) as an antiviral agent. Recent evidence for polypeptide cleavages during the replication of bacteriophages¹⁰ and many animal viruses⁹, including RNA tumour viruses¹¹, together with the results presented here, suggest that protease inhibitors¹²⁻¹⁴, or zinc may be useful in blocking certain stages of replication of many kinds of viruses.

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- ¹ Schleicher, J. B., Aquino, F. A., Rueter, A., Roderick, W. R., and Appell, R. N., *Abstr. Tenth Interscience Conf. Anti-microbial Agents and Chemotherapy*, Chicago, Abstr. No. 99 (1970).
- ² Matsumoto, S., Stanfield, F. J., Goore, M. Y., and Haff, R. F., *Proc. Soc. exp. biol. Med.*, **139**, 455 (1972).
- ³ Vallee, B. L., and Wacker, W. E. C., in *The Proteins* (edit. by Neurath, H.), **5**, No. 22, 143 (Academic Press, New York, 1972).
- ⁴ Hughes, M. N., *The Inorganic Chemistry of Biological Processes* (Wiley, London, 1972).
- ⁵ Korant, B. D., Lonberg-Holm, K., Noble, J., and Stasny, J. T., *Virology*, **48**, 71 (1972).
- ⁶ Medappa, K. C., McLean, C., and Rueckert, R. R., *Virology*, **44**, 259 (1971).
- ⁷ McLean, C., and Rueckert, R. R., *J. Virol.*, **11**, 341 (1973).
- ⁸ Butterworth, B. E., *Virology*, **56**, 439 (1973).
- ⁹ Reviewed by Sugiyama, T., Korant, B. D., and Lonberg-Holm, K. K., *A. Rev. Microbiol.*, **26**, 467 (1972).
- ¹⁰ Laemmli, U. K., *Nature*, **227**, 680 (1970).
- ¹¹ Vogt, V. M., and Eisenman, R., *Proc. natn. Acad., Sci. U.S.A.*, **70**, 1734 (1973).
- ¹² Korant, B. D., *J. Virol.*, **10**, 751 (1972).
- ¹³ Summers, D. F., Shaw, E., Stewart, M. L., and Maizel, J. V., *J. Virol.*, **10**, 880 (1972).
- ¹⁴ Korant, B. D., *J. Virol.*, **12**, 556 (1973).

Genetic evidence for SV40 gene function in enhancement of replication of human adenovirus in simian cells

HUMAN adenoviruses undergo a complete cycle of replication in human cells, but cause an abortive infection in simian cells. In the simian cells infected with the human adenovirus, early adenovirus-specific T antigen is induced^{1,2}, viral DNA is replicated to the same level as in productive cycles of replication^{3,4}, and no change in the synthesis of viral mRNA can be detected^{5,6}. However, only limited amounts of viral capsid proteins are made in relatively few cells^{1,2,6-8}. If the simian cells are coinfecting with simian virus 40 (SV40), the nonproductive cycle of replication of human adenoviruses is changed to a productive cycle^{9,10}. This helper effect of infection by SV40 is thought to be under the control of SV40 gene function, but there has been no direct evidence for this. Using temperature-sensitive (*ts*) mutants of SV40, I have now found such evidence.

The isolation and characterisation of (*ts*) mutants of SV40 have been described already^{11,12} and the properties of the mutants are summarised in Table 1. In simian cells,

TABLE 1 Properties of the SV40 temperature-sensitive mutants

Complementation groups	I	II	III
Mutant	<i>ts662</i>	<i>ts663</i>	<i>ts640</i>
Adsorption and uncoating	+	+	+
Synthesis of T antigen	+	+	ND
Synthesis of viral DNA	+	+	-
Synthesis of virion antigen	+	-	*
Infectious virus	-	-	-
Morphological transformation			
Initiation	+	+	-
Maintenance	+	+	+
Heat stability of virus particles produced at permissive temperature	Reduced	Reduced	ND
Helper function for replication of human adenovirus in simian cells†	+	+	-

Data are taken from the results of the previous studies of Kimura and Dulbecco^{11,12}, unless otherwise specified.

+, Normal at nonpermissive temperature (39°-40° C); -, restricted at non-permissive temperature; ND, not determined.

* My unpublished observations.

† This study.

these mutants replicate well at the permissive temperature of 33° C but not, if at all, at the nonpermissive temperature of 40° C, while wild type parent viruses can replicate equally well at 33-40° C. Genetic analyses have so far revealed three cistrons in SV40 genome and representative mutants of each of three complementation groups (groups I-III) were used in the study described here (Table 1). Human adenovirus type 2 (Ad2) (supplied by Dr K. Fujinaga, Aichi Cancer Centre, Nagoya) was used because it can replicate relatively well at 33°-40° C and because it can be assayed relatively easily in the indicator cells used (fl-8 cells). The stocks of Ad2 were prepared in secondary or tertiary cultures of human embryonic kidney (HEK) cells after the virus had been plaque-cloned twice in HEK cells. The stocks of SV40 and its *ts* mutants were prepared in BSC-1 cells (subline P66) as previously described¹¹. Cultures of singly or doubly infected African green monkey kidney (AGMK) cells were collected for determination of infectivity 42 h (40° C) and 88 h (33° C) after inoculation. The yield of virus in these conditions represents a single cycle of virus development for both SV40 (ref. 11 and 12 and my unpublished observations) and Ad2 (ref. 13 and my unpublished observations).

Table 2 (experiment 1) lists the yield of Ad2, parental SV40, and *ts* mutants of SV40 of complementation groups I and II in the singly or doubly infected cultures of the CV-1 line of AGMK cells at 33° C and 40° C. Clearly the *ts* mutants of SV40 of groups I and II can enhance the replication of Ad2 at 33°-40° C with efficiencies comparable with those in parental SV40-infected cells at 33°-40° C. Essentially similar results were obtained using tertiary cultures of AGMK (Table 2, experiment 3). As it has been suggested that the genes identified in these mutants (cistrons I and II) probably code for SV40 virion proteins¹¹, my results indicate that the function of the SV40 virion proteins specified by these two cistrons is not necessary for the replication of human adenovirus in simian cells. Jerkofsky and Rapp¹⁴ have also shown that other SV40 *ts* mutants, which are probably in the same group as my group I, can help the adenovirus replication in simian cells at the nonpermissive temperature. In confirmation of the previous observations^{7,15}, infection by Ad2 in turn interferes to a great extent with the replication of SV40 in the simian cells (Table 2, experiment 1).

The results of mixed infections with Ad2 and an SV40 *ts* mutant (*ts640*) of complementation group III are shown in Table 2 (experiments 2 and 3). Clearly this mutant fails to enhance the replication of Ad2 at 40° C but not at 33° C both in tertiary cultures of AGMK and in cultures

TABLE 2 Replication of adenovirus type 2 in various AGMK cells coinfecting with SV40 or *ts* mutants of SV40

Experiment No. Cells	Virus	Input multiplicities (p.f.u./per cell)	Ad2 titre (p.f.u. ml ⁻¹)			SV40 titre (p.f.u. ml ⁻¹)		
			Background (33°C 15h)	33°C 88h	40°C 42h	Background (33°C 15h)	33°C 88h	40°C 42h
(1) CV-1 line of AGMK	Ad2	8	1.5×10^4	7.2×10^2	2.0×10^3	1.0×10^5	1.5×10^8	1.0×10^7
	SV40	10						
	Ad2 + SV40	4 + 5						
	<i>ts</i> 662 (group I)	37						
	Ad2 + <i>ts</i> 662	4 + 18						
	<i>ts</i> 663 (group II)	45						
(2) Tertiary cultures of AGMK	Ad2 + <i>ts</i> 663	4 + 22	9.5×10^2	1.2×10^3	4.3×10^3	1.7×10^3	3.3×10^6	3.2×10^2
	Ad2	3						
	SV40	4						
	Ad2 + SV40	3 + 4						
	<i>ts</i> 640 (group III)	8						
	Ad2 + <i>ts</i> 640	3 + 8						
BSC-1 line of AGMK	Ad2	3	9.5×10^2	8.0×10^3	1.3×10^4	1.2×10^3	1.9×10^7	7.8×10^4
	SV40	4						
	Ad2 + SV40	3 + 4						
	<i>ts</i> 640 (III)	8						
	Ad2 + <i>ts</i> 640	3 + 8						
(3) Tertiary cultures of AGMK	Ad2	9	1.4×10^2	1.7×10^2	1.5×10^2	3.0×10^1	1.6×10^5	2.8×10^5
	SV40	4						
	Ad2 + SV40	9 + 4						
	<i>ts</i> 662 (I)	7						
	Ad2 + <i>ts</i> 662	9 + 7						
	<i>ts</i> 663 (II)	7						
	Ad2 + <i>ts</i> 663	9 + 7						
	<i>ts</i> 640 (III)	5						
	Ad2 + <i>ts</i> 640	9 + 5						

For the experiment 1, confluent monolayer cultures of CV-1 line of AGMK containing about 2×10^6 cells per 5-cm glass Petri dish were singly or doubly inoculated with 0.5 ml of Ad2, parental SV40 (strain SV40-1), or SV40 *ts* mutants, *ts* 662 (complementation group I) or *ts* 663 (group II). After adsorption at 33°C for 1 h, cultures were washed three times with medium, covered with 4 ml of Dulbecco-modified Eagle's medium containing 5% calf serum and 50 Uml⁻¹ of mycostatin, and incubated at 33°C $\pm$ 1°C or 40°C $\pm$ 0.5°C in humidified incubators flushed with a CO₂-air mixture. At specified times, cells were collected with medium (two cultures for each point) and stored frozen. After being frozen-thawed more than five times, virus infectivity in the lysates was assayed by plaque formation on cultures of fl-8 line of AGMK transformed by adeno 7-SV40 hybrid virus (E46⁺) (manuscript in preparation) at 37°C (Ad2) and on cultures of BSC-1 line (subline B) of AGMK at 33°C (SV40 and its *ts* mutants). In experiments 2 and 3, confluent 5-cm (experiment 2) or 3-cm (experiment 3) plastic Petri dish cultures containing about 2×10^6 or 7×10^5 cells, respectively, of tertiary AGMK cells or BSC-1 line (subline P66) of AGMK were singly or doubly inoculated with virus. The parental SV40 used was the strain SV68C. After adsorption at 33°C for 1.5 h cultures were treated with anti-SV40 rabbit immune serum for 30 min to remove unadsorbed SV40 and *ts* mutants of SV40 followed by two washings with medium. Cultures were covered with 5 ml (experiment 2) or 2 ml (experiment 3) of Dulbecco-modified Eagle's medium containing 10% foetal bovine serum and 50 Uml⁻¹ of mycostatin. Incubation and collection were the same as in the experiment 1. Virus-infectivity was determined by plaque formation on tertiary cultures of human embryonic kidney cells at 37°C in the presence of anti-SV40 antiserum (Ad2) and on cultures of BSC-1 line (subline B) of AGMK at 33°C (SV40 and *ts* mutants of SV40).

of BSC-1 line (subline P66) of AGMK. This strongly indicates that the SV40 gene controls, either directly or indirectly, the replication of human adenovirus in simian cells. Jerkofsky and Rapp¹⁴ reported that another SV40 early mutant (*ts*47) can enhance the adenovirus replication at the restrictive temperature. Whether these two early mutants, *ts*47 and my *ts*640, represent different genes remains to be seen. Since there has been a suggestion that the helper function is correlated with the ability of the cells to be induced to synthesise cellular DNA by infection with SV40¹⁴, it will be interesting, in a future study, to determine whether SV40 and *ts*640 induce cellular DNA synthesis in BSC-1 cells as well as in tertiary AGMK cells. In experiment 2 of Table 2, and occasionally in other experiments, the inhibition of replication of *ts*640 in a single infection of BSC-1 (P66) cells at 40°C was incomplete, in contrast to the complete inhibition in parallel experiments in tertiary AGMK cells (Table 2, experiment 2) or in CV-1 cells (my unpublished observations), suggesting the possibility, among other things, that the cistron III product of SV40 is modified by some cellular factor to carry out its function.

The question is raised: what is the nature of the SV40 gene function identified in mutant *ts*640? A previous study¹² showed that the affected gene was responsible for the synthesis of infectious viral DNA in cells of the BSC-1 line (subline P66) of AGMK and also for the initiation but not for the maintenance of morphological transformation of rat 3Y1 cells. It can be argued that *ts*640 is a double (or triple) mutant but otherwise the gene product affected has three apparently different functions, one in rat cells for the initia-

tion of transformation and the other two in simian cells for the replication of viral DNA and for the facilitation of cell infection by adenovirus. The precise roles of the gene function in each situation and accordingly the relationship between the situations are uncertain. The SV40 mutant defective in these functions described here should prove useful both in understanding adenovirus defectiveness and in defining specific mechanism controlling SV40 DNA replication and cell transformation.

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- Feldman, L. A., Butel, J. S., and Rapp, F., *J. Bact.*, **91**, 813 (1966).
- Malmgren, R. A., Rabson, A. S., Carney, P. G., and Paul, F. J., *J. Bact.*, **91**, 262 (1966).
- Rapp, F., Feldman, L. A., and Mandel, M., *J. Bact.*, **92**, 931 (1966).
- Reich, P. R., Baum, S. G., Rose, J. A., Rowe, W. P., and Weissman, S. M., *Proc. natn. Acad. Sci. U.S.A.*, **55**, 336 (1966).

- ⁵ Baum, S. G., Wiese, W. H., and Reich, P. R., *Virology*, **34**, 373 (1968).
⁶ Lucas, J. L., and Ginsberg, H. S., *J. Virol.*, **10**, 1109 (1972).
⁷ Friedman, M. P., Lyons, M. J., and Ginsberg, H. S., *J. Virol.*, **5**, 586 (1970).
⁸ Henry, C. J., Slifkin, M., and Merkov, L., *Nature new Biol.*, **233**, 39 (1971).
⁹ Rabson, A. S., O'Connor, G. T., Berezsky, I. K., and Paul, F. J., *Proc. Soc. exp. biol. Med.*, **116**, 187 (1964).
¹⁰ Beardmore, W. B., Havlick, M. J., Serafini, A., and McLean, I. W., jun., *J. Immun.*, **95**, 422 (1965).
¹¹ Kimura, G., and Dulbecco, R., *Virology*, **49**, 394 (1972).
¹² Kimura, G., and Dulbecco, R., *Virology*, **52**, 529 (1973).
¹³ Jerkofsky, M., and Rapp, F., *J. Virol.*, **2**, 670 (1968).
¹⁴ Jerkofsky, M., and Rapp, F., *Virology*, **51**, 466 (1973).
¹⁵ O'Connor, G. T., Rabson, A. S., Berezsky, I. K., and Paul, F. J., *J. natn. Cancer Inst.*, **31**, 903 (1963).

No crossing over between closely linked tumour virus loci in the heterogametic sex of fowl

RECESSIVE autosomal genes, a^r , b^r and c^r , control the resistance of fowl to Rous sarcoma virus (RSV) of subgroups A, B and C respectively¹⁻⁶. Evidence of a complete or an incomplete linkage between the tumour virus a (tva) and tumour virus c (tvc) loci has been reported⁷ and studies on an F2 population derived from a cross between C/O (chickens susceptible to subgroup A and subgroup C tumour viruses), and C/AC

The F1 parents of $a^r a^r c^r c^r$ genotype were derived from a cross between the C/O and C/AC parents. Two test cross (TC) matings were set up. In one, a double dominant heterozygote $a^r a^r c^r c^r$ F1 male was back-crossed with eight double recessive homozygote, $a^r a^r c^r c^r$ females (TC₁) and in the other eight $a^r a^r c^r c^r$ F1 females were backcrossed with one $a^r a^r c^r c^r$ male (TC₂). A detailed report on these genotypes and the development of the F1 and C/AC populations is published elsewhere⁸.

Secondary monolayer cultures of chick embryo fibroblast (CEF) from 11-d-old embryos were individually challenged in pairs with BS-RSV of subgroup A, RSV (RAV49) of subgroup C, and an equal mixture of the two viruses in the same inoculum. Plates were challenged with 0.2 ml of virus suspension diluted to contain 1000 f.f.u. ml⁻¹. Ten days after challenge foci were counted to ascertain resistant and susceptible phenotypes. The detailed procedure of recognition of the four possible subclass phenotypes within dam families and on a population basis, namely C/A (chickens resistant to subgroup A but susceptible to subgroup C); C/C, (chickens resistant to subgroup C but susceptible to subgroup A); C/O, (chickens susceptible to both subgroups) and C/AC, (chickens resistant to both subgroups), is published elsewhere⁸. In designating the phenotypes, the cultures with a focus count of 15 or less were allocated to the resistant class and those above 15 were assigned to the susceptible class.

The distribution of the four subclass phenotypes within dam families and on a population basis for the TC₁, TC₂ and F2 is shown in Table 1. Based on the observed and expected four subclass phenotype frequency ratios, that is,

TABLE 1 Distribution of four subclass phenotypes within dam families and on a population basis for TC₁, TC₂ and F2 cultured cells inoculated with BS-RSV, RSV(RAV49) and an equal mixture of the two viruses

Pheno- type	TC ₁ (F1 ♂ × C/AC ♀ ♀) (a ^a a ^r c ^r c ^r × a ^a a ^r c ^r c ^r)								Mating TC ₂ (F1 ♀ ♀ × C/AC ♂) (a ^a a ^r c ^r c ^r × a ^a a ^r c ^r c ^r)								F ₂ (F1 ♂ × F1 ♀ ♀) (a ^a a ^r c ^r c ^r × a ^a a ^r c ^r c ^r)								Total pheno- type		
	Dam No.								Dam No.								Dam No.										
	20	21	22	23	24	25	26	27	270	271	272	273	274	275	276	277	270	271	272	273	274	275	276	277			
C/O	3	4	4	1	3	4	3	2	24	5	6	1	2	3	3	6	—	26	8	9	4	6	4	6	8	7	52
C/A	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	—	0	1	0	1	0	0	0	0	0	2
C/C	0	2	2	0	1	2	1	0	8	0	0	0	0	0	0	0	—	0	0	0	2	0	2	0	0	0	4
C/AC	3	5	0	3	3	2	2	1	19	2	3	0	0	1	4	2	—	14	1	1	3	3	1	3	2	3	17
Total	7	11	6	4	7	8	6	3	52	7	9	1	2	4	7	8	—	40	10	10	10	9	7	9	10	10	75

(chickens resistant to both subgroups) parents favoured incomplete linkage of these two loci⁸. The linkage value computed from the results of the F2 population was 0.08 ± 0.03 based on an assumption of equal crossing over in gametes produced by the male and female parents. This report describes the distribution of segregants arising from two reciprocal double back crosses (F1 ♂ × C/AC ♀ ♀ and F1 ♀ × C/AC ♂), which strongly suggests the absence of crossing over in the heterogametic sex (female).

1 C/O: 1 C/A: 1 C/C: 1 C/AC: the χ^2 analysis⁹ for the TC₁ is shown in Table 2. A non-random segregation and re-association of the genes at the tva and tvc loci was indicated by the highly significant χ^2 value, 25.08 ($P < 0.01$). The single degree χ^2 analysis⁹ suggested linkage between the tva and tvc loci, because the segregations of $a^r:a^r$ and $c^r:c^r$ genes were not significant ($P > 0.05$) in contrast to a highly significant χ^2 value, 22.23 ($P < 0.01$), for the joint segregation component (Table 2).

TABLE 2 χ^2 linkage test for detection of linkage between tva and tvc loci in the TC₁ population and segregation analysis of the phenotypes based on $\dagger p = 0.5$ and 0.17

Genotype	Phenotype	Observed number	Expected frequency	Expected No.		χ^2 deviation		χ^2 linkage test $p = 0.5$	d.f.	χ^2
				$p = 0.5$	$p = 0.17$	$p = 0.5$	$p = 0.17$			
$a^r a^r c^r c^r$	C/O	24	$\frac{1}{2}(1-p)$	13.00	21.32	9.31	0.02	segregation, $a^r:a^r$	1	2.77*
$a^r a^r c^r c^r$	C/A	1	$\frac{1}{2}(p)$	13.00	4.68	11.08	2.89	segregation, $c^r:c^r$	1	0.08*
$a^r a^r c^r c^r$	C/C	8	$\frac{1}{2}(p)$	13.00	4.68	1.92	2.36	joint segregation (linkage)	1	22.23†
$a^r a^r c^r c^r$	C/AC	19	$\frac{1}{2}(1-p)$	13.00	21.32	2.77	0.25			
Total		52	1.00	52.00	52.00	25.08†	5.52*		3	25.08†

* Significance level; $P > 0.05$.

† Significance level; $P < 0.01$.

‡ $p = 0.5$ that is, random reassociation of non-allelic pairs of genes at the tva and tvc loci.

TABLE 3 χ^2 linkage test for the detection of linkage between the *tva* and *tvc* loci in the TC₂ population and segregation analysis of the phenotypes based on $p = 0.5, 0.17$ and 0.00

Genotype	Phenotype	Observed No.	Expected frequency	Expected No.			χ^2 deviation			χ^2 linkage test	
				$p = 0.5$	$p = 0.17$	$p = 0.00$	$p = 0.5$	$p = 0.17$	$p = 0.00$	$p = 0.5$	$p = 0.5$
$a^+a^+c^+c^+$	C/O	26	$\frac{1}{2}(1-p)$	10	16.60	20.00	25.60	5.32	1.80	Segregation $a^+:a^-$	d.f. χ^2
$a^+a^+c^-c^-$	C/A	0	$\frac{1}{2}(p)$	10	3.40	0	10.00	3.40	0	Segregation $c^+:c^-$	1 3.60*
$a^-a^-c^+c^+$	C/C	0	$\frac{1}{2}(p)$	10	3.40	0	10.00	3.40	0	Joint segregation (linkage)	1 40.00†
$a^+a^+c^-c^-$	C/AC	14	$\frac{1}{2}(1-p)$	10	16.60	20.00	1.60	0.41	1.80		
Total		40	1.00	40	40.00	40.00	47.20†	12.53†	3.60*		3 47.20†

* Significance level; $P > 0.05$.† Significance level; $P < 0.01$.

Nine out of fifty-two were cross over phenotypes (C/A + C/C), and by the maximum likelihood method⁹ the linkage value (p) was estimated to be 0.17 ± 0.05 . On the basis of this p value, the observed phenotype frequencies did not deviate significantly from those of the expected ones ($\chi^2 = 5.52$, $P > 0.05$, Table 2).

The χ^2 analysis for the TC₂, based on observed and expected four subclass phenotypes in a ratio of 1:1:1:1, also gave strong support for linkage between the *tva* and *tvc* loci (Table 3). No cross-over phenotype of either C/A or C/C, out of 40 embryos tested was observed however. It is also possible that very little crossing over had occurred in the TC₂, and that by chance the cross-over products did not appear in this population. This supposition is not testable as p is unknown. Under the assumption of equal p in either TC, that is, $p = 0.17 = TC_1 = TC_2$, the χ^2 value, 12.53 ($P < 0.01$) significantly rejected this postulate (Table 3). Alternatively on the basis of $p = 0.00$, the χ^2 analysis presented in Table 3 favoured the observed 1:1 segregation ($\chi^2 = 3.60$, and $P > 0.05$), which suggests the absence of crossing over in the TC₂.

In the absence of linkage between the *tva* and *tvc* loci, four types of gametes, a^+c^+ , a^+c^- , a^-c^+ and a^-c^- should have been produced by male and female parents at equal frequencies during gametogenesis in the TC₁ and TC₂ respectively. But because of linkage between the two loci the expected frequency distribution of the gametic array was disturbed in proportion to the crossing over between the non-allelic pairs of genes. As a result of differential crossing over in the gametes produced by double heterozygote males of the TC₁ and females of the TC₂, 17% of crossing over was observed in the TC₁ population compared to none in the TC₂. These results and their appropriate χ^2 analysis strongly imply the absence of crossing-over in the heterogametic sex.

This concept was further analysed on the results of the distribution of subclass phenotypes of the F2 population reported earlier⁸. The observed and expected segregation of the four subclass phenotypes of the F2 population is shown in

Table 4. With $p = 0.5$, the χ^2 value was highly significant ($P < 0.01$), indicating a non-random reassociation of genes at the *tva* and *tvc* loci and the linkage test shown in Table 4 confirmed that the *tva* and *tvc* loci are linked ($\chi^2 = 51.81$, $P < 0.01$). In our previous report the results were analysed under the assumption of an equal crossing over in the male and female gametes and the χ^2 analysis supported this concept. Accordingly the p value was estimated to be 0.08 ± 0.03 . The predicted frequency distribution of the four subclass phenotypes, C/O, C/A, C/C and C/AC based on the p value estimated in this study, (0.17 for male and 0.00 for female) is also presented in Table 4. Because the χ^2 value, 0.86 was not significant ($P > 0.05$), the proposed concept of the absence of crossing over in the heterogametic sex of fowl is upheld. The overall p value 0.10 ± 0.03 estimated from the two reciprocal double back crosses, agreed well with that reported previously and by combining the data of the TC₁, TC₂ and F2 populations the best joint estimate of p was 0.09 ± 0.02 . Results obtained from other crosses also support the present findings and will be published elsewhere.

Thus from the results of TC₁, TC₂ and F2 two important conclusions can be drawn: (1) the *tva* and *tvc* loci are linked with a linkage value of 0.17 ± 0.05 , (2) very little or no crossing over occurs between the *tva* and *tvc* loci in the heterogametic sex.

It is an established hypothesis that in many species the heterogametic sex has a strong influence on crossing over during gametogenesis¹⁰. In *Drosophila* the mutant, *c(3)G* in chromosome III has been shown to suppress crossing over in the male¹²; but it is not known whether similar mutants exist in other species.

In fowl, however, sex has little effect on crossing over in all linkage groups except the rose comb-creeper pair¹⁰⁻¹¹. In the latter, crossing over was observed more than twice as frequently in the females as in the males. The results in this study on the other hand, suggest the contrary because very little or no crossing over occurred between the *tva* and *tvc* loci in the female compared with 17% in the male. Because in the fowl the female is the heterogametic sex, the result

TABLE 4 χ^2 linkage test for the detection of linkage between the *tva* and *tvc* loci in the F2 population and segregation analysis of phenotypes based on $p\sigma = 0.17$ and $p\varphi = 0.00$

Genotype	Phenotype	Observed No.	Expected frequency		Expected No.		χ^2 deviation		χ^2 linkage test	
			$p = 0.5$	$p\sigma = 0.17$ $p\varphi = 0.00$	$p = 0.5$	$p\sigma = 0.17$ $p\varphi = 0.00$	$p = 0.5$	$p\sigma = 0.17$ $p\varphi = 0.00$	$p = 0.5$	$p = 0.5$
a^+c^+--	C/O	52	0.5625	0.7050	42.187	52.875	2.28	0.01	Segregation, $a^+:a^-$	d.f. χ^2
$a^+a^+c^+--$	C/A	2	0.1875	0.0450	14.063	3.375	10.35	0.56	Segregation, $c^+:c^-$	1 0.00*
$a^+a^+c^-c^-$	C/C	4	0.1875	0.0450	14.063	3.375	7.20	0.12	Joint segregation (linkage)	1 0.36*
$a^+a^+c^-c^-$	C/AC	17	0.0625	0.2050	4.687	15.375	32.34	0.17		1 51.81†
Total		75	1.0000	1.0000	75.000	75.000	52.17†	0.86*		3 52.17†

* Significance level; $P > 0.05$.† Significance level; $P < 0.01$.

in this study therefore supports the present hypothesis that heterogametic sex influences crossing over. In view of the inconsistent sex effect in other linkage groups an explanation of the sex influence on crossing over in the *tva-tvc* linkage pair is difficult without further studies. It may be that the F1 females of the strain of fowl under study are carrying structural chromosomal changes, such as inversion and translocation, which could influence crossing over indirectly¹². A careful cytological study might show such changes.

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- ¹ Crittenden, L. B., and Okazaki, W., *J. natn. Cancer Inst.*, **35**, 857 (1965).
- ² Rubin, H., *Virology*, **26**, 270 (1965).
- ³ Payne, L. N., and Biggs, P. M., *Virology*, **29**, 190 (1966).
- ⁴ Payne, L. N., and Biggs, P. M., *J. gen. Virol.*, **7**, 177 (1970).
- ⁵ Crittenden, L. B., Stone, H. A., Reamer, R. H., and Okazaki, W., *J. Virol.*, **1**, 898 (1967).
- ⁶ Motta, J. V., Crittenden, L. B., and Pollard, W. O., *Poult. Sci.*, **52**, 578 (1973).
- ⁷ Payne, L. N., and Pani, P. K., *J. gen. Virol.*, **13**, 253 (1971).
- ⁸ Pani, P. K., *J. gen. Virol.*, **22**, 187 (1974).
- ⁹ Mather, K., *The measure of linkage in heredity* (Methuen, London, 1963).
- ¹⁰ Hutt, F. B., *Genetics of the fowl* (McGraw-Hill, London, 1949).
- ¹¹ Tayler, L. W., *J. Hered.*, **25**, 205 (1934).
- ¹² Swanson, C. P., Merz, T., and Young, W. J., *Cytogenetics* (Prentice-Hall, Englewood Cliffs, New Jersey, 1967).

Scrapie in sheep selectively bred for high susceptibility

THE enigma of scrapie disease of sheep is still unresolved, in spite of intensive efforts—especially over the past 12 yr—to elucidate its pathogenesis and to define the transmissible agent. Various hypotheses on the nature of scrapie remain unproved¹⁻¹⁰.

It has long been recognised that heredity is an important factor in the transmission of naturally-occurring scrapie. In 1961 a selective breeding programme with Herdwick sheep was started at this Institute, with the objective of developing from the same foundation stock, two flocks, one highly

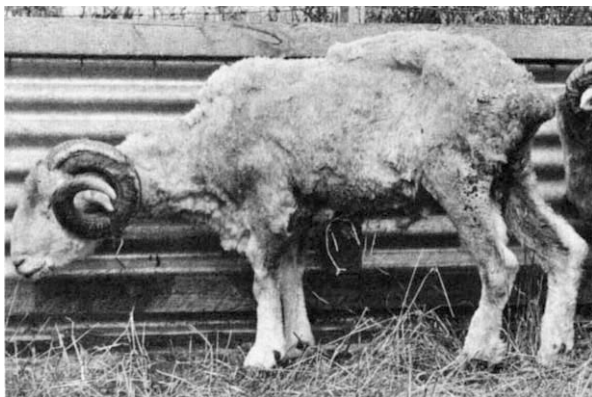


FIG. 1 Case A: a scrapie-affected ram. Note dullness, rubbed areas and hind-limb weakness.

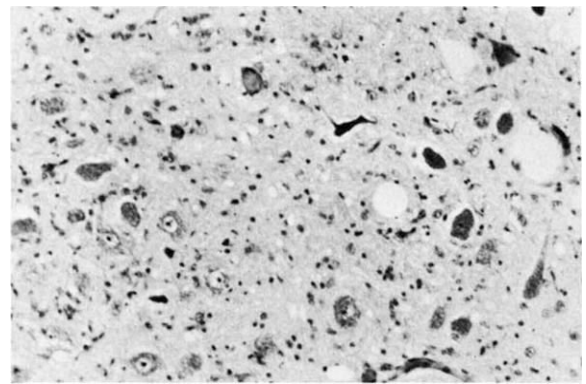


FIG. 2 Case A: a histological section of the medulla oblongata. There is extensive spongiform degeneration of the 10th nucleus. Stained with haematoxylin and eosin (X25).

susceptible and the other highly resistant to experimental scrapie. This objective has now been achieved¹¹. Briefly, in two successive years 40 rams and 699 ewes were mated in forty separate flocks to produce two crops of lambs. One crop of lambs and their parents were then challenged with scrapie by inoculation. A proportion of the inoculated animals developed scrapie during the following 2 yr. The non-inoculated crop of offspring could then be defined in terms of the susceptibility to scrapie of their parents and their sibs. Animals of which the parents and sibs developed scrapie were the foundation stock for the present flock that is highly susceptible to experimental scrapie; likewise, the foundation stock of the highly resistant flock had parents and sibs that did not develop scrapie.

The breeding part of this programme has been carried out exclusively in five buildings well separated from each other on a restricted and clearly defined experimental area of the 2,000 acre estate that is part of this Institute. Neither natural nor experimental scrapie had ever been present in this area before the occurrence of the two cases of the disease described here. Sheep to be inoculated with scrapie have always been removed to one of two other clearly defined areas on the estate, each approximately 1 mile away. Different shepherds have looked after the breeding flocks and infected flock, respectively. The isolation of the breeding flocks has been strictly maintained.

Both the cases of scrapie that are the subject of this communication have occurred in the flock that has been bred for high susceptibility to experimental scrapie. The first (case A) was in July 1972 in a ram 40 months old. Clinical signs of scrapie were typical, and included rubbing over the rump, hind-limb incoordination and weakness, and a dull facial expression (Fig. 1). The second (case B) was in November 1973 in a ewe 43 months old. Again, the clinical signs were typical and closely similar to those of case A. Both animals were observed for a few days and were then killed for *post mortem* examination. In both cases histological examination of the brain showed in the medulla oblongata the characteristic bilaterally symmetrical spongiform encephalopathy of scrapie, with vacuolation of the neurones; spongiform change was particularly marked in the region of the 10th nucleus (Fig. 2). Neurones showing foamy vacuolation (Fig. 3) were present in both cases. This type of vacuolation is typical of natural scrapie, and is rare in the experimentally produced disease¹². Also, in both cases vacuolated neurones were more obvious and more numerous than is usual in the experimental disease^{12,13}.

Because the histopathology of these two cases was that of naturally-occurring scrapie, a histological re-examination was made of the 99 cases of scrapie that have occurred in

Herdwick sheep inoculated at this Institute over the past 6 yr with a standard dose of a pool of homogenised scrapie sheep brain. A single section of the medulla oblongata close to the calamus scriptorius¹⁴ from each of these 99 brains was compared with single sections of the same area from cases A and B. Particular attention was paid to vacuolated neurones, foamy vacuolation of neurones and spongiform vacuolation of the 10th nucleus. The results are shown in Table 1. These results indicated strongly that the scrapie of cases A and B differed from the experimental scrapie produced by the standard inoculum. Because scrapie can still be defined only in clinicopathological terms, no other test was available to differentiate between these two expressions of the disease. It may be noted, however, that both cases occurred at ages (40 and 43 months) close to the mean of 39 months in the highly characteristic age incidence pattern recorded by Parry² for 1,008 cases of naturally-occurring scrapie.

Scrapie cases A and B both occurred in the flock bred for high susceptibility to experimental scrapie. Although they had four common ancestors, they were not very closely related to each other. The coefficient of inbreeding¹⁵ of case A was 0.063, and of case B was 0.008.

The mechanism of the spread of scrapie disease has been extensively studied.¹⁶ It is generally accepted that natural scrapie is usually perpetuated in a hereditary manner, and the very rare exceptions in which there has apparently been lateral spread may have been related to ingestion of foodstuff contaminated by foetal membranes voided by a scrapie-affected ewe at lambing time¹⁷. The experimental disease does not spread by contact, unless—as may happen with rodents—there has been ingestion of scrapie tissue. It is extremely unlikely, therefore, that scrapie cases A and B could have occurred by either direct or indirect contact with natural or experimental scrapie. They were separated in time by 16 months, and the physical organisation of the experimental programme makes it impossible that they were accidentally inoculated with scrapie; even had this been so, the histopathology would have been that of the experimental disease.

I conclude that these two cases of scrapie arose spontaneously by genetic selection in a breeding programme designed to increase susceptibility to experimental scrapie. This conclusion supports the proposition put forward by Parry² that in the naturally-occurring disease the scrapie agent can be formed within susceptible sheep, and it agrees with the long-established fact that scrapie is frequently associated with attempts to improve the conformation and performance of sheep by inbreeding¹⁸.

A genetic basis for the development of scrapie in sheep is consistent with observed facts, but it leaves unresolved the problem of the nature of the transmissible agent. A majority of investigators have assumed that the agent is self-replicating because its titre in body tissues rises during sub-clinical and clinical development of the disease. Self-replication implies involvement of nucleic acid. Nucleic acid, however, has not been identified, and there is impressive experimental evidence from ultraviolet irradiation studies

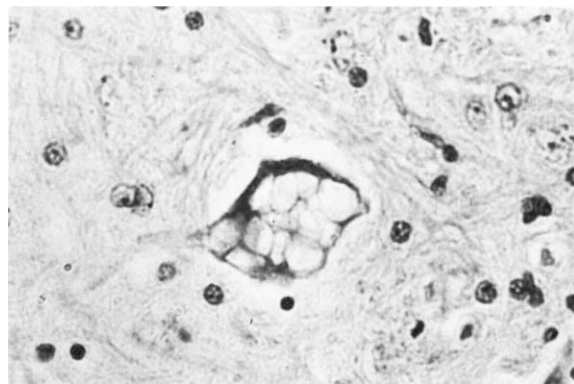


FIG. 3 Case B: a histological section of the medulla oblongata showing foamy vacuolation of a neurone. Stained with haematoxylin and eosin (X105).

that it may not be present in the agent¹⁹⁻²². Pattison and Jones⁵ pointed out that if the scrapie agent were present in normal tissues in an inhibited form and if it were gradually released as scrapie developed, this unmasking would provide an alternative explanation to self-replication. It seems possible that a mechanism such as this could be genetically controlled, and that resultant disorganisation of metabolism could cause degeneration of the central nervous system.

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- ¹ Cuillé, J., and Chelle, P. L., *C. r. hebdom. Séanc. Acad. Sci., Paris*, **206**, 1687 (1933).
- ² Parry, H. B., *Heredity, Lond.*, **17**, 75 (1962).
- ³ Pattison, I. H., and Jones, K. M., *Vet. Rec.*, **80**, 2 (1967).
- ⁴ Gibbons, R. A., and Hunter, G. D., *Nature*, **215**, 1041 (1967).
- ⁵ Pattison, I. H., and Jones, K. M., *Nature*, **218**, 102 (1968).
- ⁶ Hunter, G. D., Kimberlin, R. H., and Gibbons, R. A., *J. theor. Biol.*, **20**, 355 (1968).
- ⁷ Field, E. J., *Int. Rev. exp. Path.*, **8**, 129 (1969).
- ⁸ Adams, D. H., *Path. Biol.*, **18**, 559 (1970).
- ⁹ Gibbs, C. J., and Gajdusek, D. C., *Proc. 7th Int. Cong. Neuropath.*, 779 (1970).
- ¹⁰ Diener, T. O., *Nature new Biol.*, **235**, 218 (1972).
- ¹¹ Nussbaum, R. E., Henderson, W. M., Pattison, I. H., Elcock N. V., and Davies, D. C., *Res. vet. Sci.* (in the press).
- ¹² Zlotnik, I., *J. comp. Path. Ther.*, **68**, 428 (1958).
- ¹³ Wilson, D. R., Anderson, R. D., and Smith, W., *J. comp. Path. Ther.*, **60**, 267 (1950).
- ¹⁴ Holman, H. H., and Pattison, I. H., *J. comp. Path. Ther.*, **53**, 231 (1943).
- ¹⁵ Li, F. H. F., and Roderick, T. H., *J. Hered.*, **61**, 37 (1970).
- ¹⁶ Pattison, I. H., *Vet. Rec.*, **76**, 333 (1964).
- ¹⁷ Pattison, I. H., Hoare, M. N., Jebbett, J. N., and Watson, W. A., *Vet. Rec.*, **90**, 465 (1972).
- ¹⁸ McGowan J. P., *Investigation into the disease of sheep called scrapie*. (William Blackwood and Sons, Edinburgh, 1914).
- ¹⁹ Alper, T., Haig, D. A., and Clarke, M. C., *Biochem. biophys. Res. Commun.*, **22**, 278 (1966).
- ²⁰ Alper, T., Cramp, W. A., Haig, D. A., and Clarke, M. C., *Nature*, **214**, 764 (1967).
- ²¹ Haig D. A., Clarke, M. C., Blum, E., and Alper, T., *J. gen. Virol.*, **5**, 455 (1969).
- ²² Latarjet, R., Muel, B., Haig, D. A., Clarke, M. C., and Alper, T., *Nature*, **227**, 1341 (1970).

TABLE 1 Comparison of single sections of the medulla oblongata from 99 cases of experimental scrapie with those from cases A and B.

Group	Number of cases showing:		
	Vacuolated neurones	Foamy vacuolation of neurones	Spongiform vacuolation of the 10th nucleus
99 cases of experimental scrapie	30	0	0
Scrapie Cases A and B	2	2	2

Anti-muscarinic properties of neuroleptics and drug-induced Parkinsonism

ONE unifying hypothesis of the behavioural effects produced by neuroleptic drugs is that they act by blocking dopamine receptors in the central nervous system¹⁻³. Neuroleptic drugs produce increases in dopamine turnover⁴, increase accumulation of its metabolites⁵, antagonise amphetamine induced behaviour in man and animals⁶ and block the stimulating effects of dopamine on cyclic AMP production in homogenates of rat brain striatum or mesolimbic nuclei⁷⁻⁹.

One complication in the use of these drugs is that they often produce severe extrapyramidal side effects including a syndrome similar to idiopathic Parkinsonism^{10,11}. This syndrome has been explained as a blockade of dopamine receptors by the drugs resulting in a functional lack of dopamine in the basal ganglia. The extrapyramidal side effects may be treated by centrally acting anticholinergic agents¹² which restore the balance between the antagonistic dopaminergic and cholinergic influences in the basal ganglia¹¹ without interfering with the antipsychotic actions of the neuroleptics.

It has been shown⁸ that a series of neuroleptics differed over a 50-fold range in their ability to inhibit the striatal dopamine-sensitive adenylyl cyclase (Table 1). The more potent antidopaminergic drugs, however, are used clinically in much lower doses than the less potent drugs and on this basis one might therefore expect all neuroleptics to produce about the same incidence of extrapyramidal signs. That this is not the case has become increasingly apparent. In particular the phenothiazine, thioridazine, and the recently described dibenzodiazepine, clozapine, have been shown to produce very few extrapyramidal motor side effects¹³⁻¹⁵. Clozapine has been shown to be an anticholinergic agent using the guinea pig ileum and it has been suggested that the paucity of extrapyramidal side effects seen with this drug was a result of this property¹⁶⁻¹⁸. In the present experiments we have used a specific radioactive affinity label for muscarinic receptors to determine if any relationship exists between drug induced Parkinsonian signs and anti-muscarinic activity in neuroleptic drugs.

Male Wistar rats were decapitated and their brains removed. The cerebral cortex was homogenised in 10 volumes of ice cold 0.32 M sucrose. Protein concentration was determined by the method of Lowry *et al.*¹⁹ and the homogenate was diluted in Krebs-Henseleit solution (equilibrated with 5% CO₂ in oxygen) to give 0.27 mg protein ml⁻¹. 3 ml aliquots of homogenate were preincubated for 15 min with the drug being tested and then the aziridinium ion from N-2'-chloroethyl-N-[2'', 3'', ³H₂]-propyl-2-aminoethylbenzi-

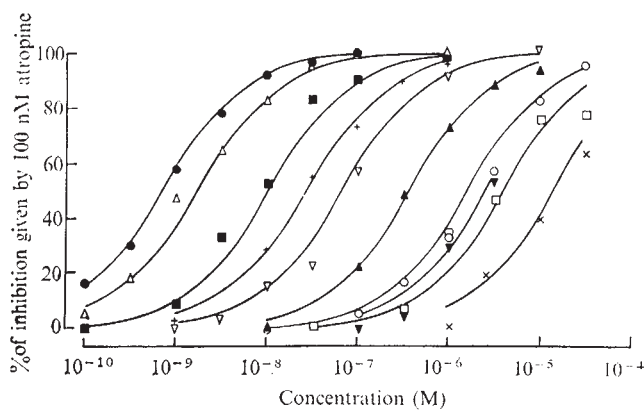


FIG. 1 Inhibition of atropine-sensitive uptake of ³H-PrBCM by neuroleptic agents and other drugs. Experiments were performed in quadruplicate and standard errors were < 10%. The lines are theoretical mass action curves derived using the dissociation equilibrium constants given in Table 1. Atropine-sensitive uptake was approximately 60% of a total uptake of 700 pmol ³H-PrBCM per g protein. ●, Atropine; Δ, benztropine; ■, ethopropazine; +, thioridazine; ▽, clozapine; ▲, chlorpromazine; ○, pimozide; ▼, flupenthixol; □, trifluoperazine; ×, spiroperidol.

late (³H-PrBCM: 1.8 Ci mmol⁻¹) added to give a final concentration of 2 × 10⁻⁹ M. The aziridinium ion was prepared as described by Burgen *et al.*²⁰. Incubation was continued for 8 min and was stopped by the addition of 20 ml Krebs-Henseleit solution containing 10 mM sodium thiosulphate. Samples were filtered under suction through Whatman GF/C glass fibre filters, washed with 20 ml of Krebs-Henseleit solution and the tritium remaining on the filters determined by liquid scintillation counting.

The concentration of ³H-PrBCM used produced 88% receptor occupation in an uninhibited reaction. Percentage inhibition of the rate constant of alkylation was determined by the method of Burgen *et al.*²¹. Dissociation equilibrium constants were derived from the slope of the plot (fractional inhibition of rate constant)⁻¹ against (concentration of reversible inhibitor)⁻¹.

The effect of different drug concentrations on the atropine-sensitive binding of ³H-PrBCM in cortical homogenates is shown in Fig. 1. Dissociation equilibrium constants for the various drugs are listed in Table 1. The most potent drug used was the classical antimuscarinic agent, atropine, which was included for comparison. It is clear that the neuroleptics differ over a wide range (approximately four orders of magnitude) in their antimuscarinic potencies. The results obtained here support the hypothesis that the antimuscarinic activity of these drugs is related to the frequency of extrapyramidal side effects. For example, in considering drugs of the phenothiazine class one finds a spectrum of effects. These drugs range from trifluoperazine, a potent dopamine receptor blocker with little antimuscarinic activity, that causes a high frequency of extrapyramidal effects, through chlorpromazine and thioridazine to ethopropazine, a drug with no neuroleptic effects, which has in fact been used as an anti-Parkinsonian agent¹¹. The antimuscarinic effect of benztropine, a classical anticholinergic, anti-Parkinsonian agent¹¹ is shown for comparison. The incidence of extrapyramidal effects found with the other drugs is also inversely related to their antimuscarinic activity. Spiroperidol, a butyrophenone and flupenthixol, a thioxanthene, have weak antimuscarinic effects and produce frequent side effects¹³. Clozapine produces few side effects and is a considerably more potent antimuscarinic agent.

Several other observations concerning the behavioural and metabolic effects of neuroleptics may also be interpreted in the light of these results. Thus, clozapine causes a smaller

TABLE 1 Dissociation equilibrium constants of various drugs for cortical muscarinic receptors

Drug	Dissociation equilibrium constant (M)	IC ₅₀ for striatal* dopamine-sensitive adenylyl cyclase (M)
Atropine	5.2 ± 0.3 × 10 ⁻¹⁰	—
Benztropine	1.3 ± 0.1 × 10 ⁻⁹	> 10 ⁻⁴
Ethopropazine	1.0 ± 0.1 × 10 ⁻⁸	—
Thioridazine	2.5 ± 0.1 × 10 ⁻⁸	3.0 × 10 ⁻⁶
Clozapine	5.5 ± 1.0 × 10 ⁻⁸	4.5 × 10 ⁻⁶
Chlorpromazine	3.5 ± 0.2 × 10 ⁻⁷	1.0 × 10 ⁻⁶
Pimozide	1.6 ± 0.1 × 10 ⁻⁷	4.5 × 10 ⁻⁶
Trifluoperazine	4.0 ± 0.4 × 10 ⁻⁶	4.0 × 10 ⁻⁷
Flupenthixol	2.2 ± 0.8 × 10 ⁻⁶	7.0 × 10 ⁻⁸
Spiroperidol	1.2 ± 0.1 × 10 ⁻⁵	2.0 × 10 ⁻⁶

The dissociation equilibrium constants are determined as in the text. The effect of some of these compounds on striatal dopamine-sensitive adenylyl cyclase is shown for comparison.

* Data from Miller and Iversen, 1974⁸.

increase in homovanillic acid accumulation in basal ganglia than in the limbic system of rabbits¹⁷ and thioridazine produces little increase in homovanillic acid accumulation in monkey basal ganglia¹. In addition, it has recently been suggested that the different effects of thioridazine and chlorpromazine in antagonising amphetamine-induced turning in rats, argue against dopamine receptor blockade being involved in neuroleptic activity²². These results, however, may be explained by the more potent antimuscarinic activity of thioridazine.

In conclusion, the results presented here support the hypothesis that neuroleptic drugs with potent antimuscarinic activity produce few Parkinsonian side effects. Combination of the method used here with an *in vitro* measure of dopamine receptor blockade, such as the dopamine-sensitive adenylyl cyclase, should ensure improved and rapid selection of potentially useful neuroleptic agents.

In the course of this study we learned of a similar series of experiments performed by Snyder, Greenberg and Yamamura²³. The results of their work seem to agree with those reported here.

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- ¹ Matthyse, S., *Fedn Proc.*, **32**, 200-205 (1973).
- ² Van Rossum, J. M., *Arch. int. Pharmacodyn.*, **160**, 492-494 (1960).
- ³ Horn, A. S., and Snyder, S. H., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 2325-2328 (1971).
- ⁴ Nyback, H., Borzecki, Z., and Sedvall, G., *Eur. J. Pharmac.*, **4**, 395-403 (1968).
- ⁵ Anden, N.-E., Roos, B.-E., and Werdinius, B., *Life Sci.*, **3**, 149-158 (1964).
- ⁶ Snyder, S. H., *Arch. gen. Psychiat.*, **27**, 169-179 (1972).
- ⁷ Kebabian, J. W., Petzold, G. L., and Greengard, P., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 2145-2149 (1972).
- ⁸ Miller, R. J., and Iversen, L. L., *Trans. Biochem. Soc.*, (in the press).
- ⁹ Horn, A. S., Cuello, A. C., and Miller, R. J., *J. Neurochem.*, (in the press).
- ¹⁰ Hornykiewicz, O., *Pharmac. Rev.*, **18**, 925-964 (1966).
- ¹¹ Klawans, H. L., *The Pharmacology of Extrapyramidal Movement Disorders. Monographs in neural sciences 2*, (edit. by Cohen, M.) (Karger, Basel, 1973).
- ¹² Freyhan, F. A., in *Extrapyramidal System and Neuroleptics* (edit. by Bordeleau, J. M.) 483-486 (Editions Psychiatriques, Montreal, 1961).
- ¹³ Klein, D. F., and Davis, J. M., *Diagnosis and Drug Treatment of Psychiatric Disorders* (Williams and Wilkins, Baltimore, 1969).
- ¹⁴ Shader, R. I., and Di Mascio, A., *Psychotropic Drug Side Effects* (Williams and Wilkins, Baltimore, 1970).
- ¹⁵ Stille, G., and Hippus, A., *Pharmacopsychiatry*, **4**, 182-191 (1971).
- ¹⁶ Anden, N.-E., *J. Pharm. Pharmac.*, **24**, 905-906 (1972).
- ¹⁷ Anden, N.-E., *J. Pharm. Pharmac.*, **25**, 346-348 (1973).
- ¹⁸ Stille, G., Lauener, H., and Eichenberger, E., *Il Farmaco*, **26**, 603-625 (1971).
- ¹⁹ Lowry, O. H., Rosebrough, N. H., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265-275 (1951).
- ²⁰ Burgen, A. S. V., Hiley, C. R., and Young, J. M., *Br. J. Pharmac.*, (in the press).
- ²¹ Burgen, A. S. V., Hiley, C. R., and Young, J. M., *Br. J. Pharmac.*, (in the press).
- ²² Crow, T. J., and Gillbe, C., *Nature*, **245**, 27-28 (1973).
- ²³ Snyder, S. H., Greenberg, D., and Yamamura, H. I., *Arch. gen. Psychiat.*, (in the press).

Inhibition of normal clotting and Fletcher factor activity by rabbit anti-kallikrein antiserum

NORMAL human plasma clots rapidly on contact with a foreign surface such as glass, through a sequence of reactions designated the intrinsic pathway of thrombin formation. Two nearly identical mechanisms, designated a waterfall¹ and cascade² respectively, were proposed to explain this process. Although these hypotheses have since been modified³, ideas about the clotting factors involved and the essential order of their participation have not changed. Many of the reactions have been clarified through the use of plasma from patients with congenital deficiencies and partially purified preparations of clotting factors.

Hathaway *et al.*⁴ described a family in whom impaired blood clotting seemed to be due to the deficiency of a hitherto unrecognised clotting factor. The defect, designated Fletcher factor deficiency or Fletcher trait, after the affected kindred, has been recognised in at least three further patients⁵. Wuepper⁶ reported that Fletcher trait plasma is functionally and antigenically deficient in a plasma prekallikrein, and that the addition of purified preparations of prekallikrein corrected the clotting defect of Fletcher trait plasma. This observation, confirmed in this and other laboratories^{7,8}, suggests that a plasma prekallikrein or its activated form, kallikrein, participates in normal blood clotting. To examine this possibility further, we have prepared antiserum in rabbits against purified human plasma kallikrein and examined its effect on the clotting of normal human plasma. We report here that this anti-kallikrein antibody impedes the intrinsic pathway of thrombin formation by depleting normal plasma of Fletcher factor activity.

Partially purified human plasma kallikrein was prepared from human oxalated plasma by tricalcium phosphate adsorption, Celite 512 adsorption and elution, and successive column chromatography on SP-Sephadex C-50, QAF-Sephadex A-50 and Sephadex G-150 (Pharmacia, New Market, New Jersey)⁹. The preparation released 26.4 μ M methyl alcohol (MeOH) ml⁻¹ h⁻¹ (specific activity: 775 μ M MeOH mg⁻¹ protein h⁻¹) from *p*-toluenesulphonyl-L-arginine methyl ester¹⁰ and approximately 250 ng of bradykinin per ml in the rat uterus assay¹¹. The kinin generating activity was inhibited by soybean trypsin inhibitor (0.5 mg ml⁻¹) and Trasylol (500 U ml⁻¹), but not by lima bean trypsin inhibitor (0.5 mg ml⁻¹). It contained traces of activated PTA activity (less than 0.005 U ml⁻¹), but no measurable amount of other known clotting factors or plasmin.

TABLE 1 Effect of anti-kallikrein antibody on screening tests of blood coagulation

Test	Clotting time	
	Normal rabbit γ G + normal plasma	Anti-kallikrein antibody + normal plasma
	(s)	(s)
Thrombin time*	31.4	32.8
One-stage prothrombin time	16.8	16.8
Russell's viper venom time	11.9	12.6
Kaolin-activated partial thromboplastin time†	67.0	298.4

A sample of 0.4 ml normal human pooled plasma was incubated with equal amounts of either normal rabbit γ G (21 mg protein per ml) or anti-kallikrein antibody (33 mg protein per ml) in a 10 $\times$ 75 mm polystyrene tube at 37° C for 60 min. The mixture was then tested. The techniques used in these tests have been summarised before¹⁵.

* 2.5 NIH U/ml crude bovine thrombin (Parke, Davis, Detroit).

† 0.1 ml test sample was incubated with 0.1 ml kaolin-Gliddex-P in a 10 $\times$ 75 mm glass tube at 37° C 1 min before adding 0.1 ml 0.025 M CaCl₂.

Rabbit antiserum to human plasma kallikrein was obtained by injecting a mixture of equal parts of partially purified kallikrein, containing approximately 50 µg protein, and alumina Cγ-gel (A grade, Calbiochem), into the footpads of New Zealand albino female rabbits. Four weeks later

TABLE 2 Effect of anti-kallikrein antibody on some clotting factors in the intrinsic pathway

Clotting factor	Dilution of anti-kallikrein or normal rabbit γG	Clotting time			
		Normal rabbit γG		Anti-kallikrein antibody	
		normal plasma	normal plasma	normal plasma	normal plasma
		(s)	(%)	(s)	(%)
Hageman factor	Undiluted	66.2	100	68.6	86
PTA	Undiluted	121.5	100	116.6	105
Christmas factor	Undiluted	120.9	100	121.5	100
AHF	Undiluted	111.5	100	112.8	100
Fletcher factor	Undiluted	103.6	100	378.2	<1
Fletcher factor	1/2	—	—	378.8	<1
Fletcher factor	1/4	—	—	335.7	<1
Fletcher factor	1/8	—	—	224.6	2.5

A sample of 0.05 ml normal human pooled plasma was incubated with equal amounts of either normal rabbit γG (21 mg protein per ml) or various dilutions of anti-kallikrein antibody (33 mg protein per ml) in 10 × 75 mm polystyrene tubes at 37°C for 60 min. Then, 1.9 ml ice-cold barbitol-saline buffer (0.025 M barbitol-sodium barbitol, 0.125 M sodium chloride, pH 7.5) was added to each tube and 0.1 ml aliquots were assayed for each clotting factor listed above. The assays of Hageman factor, PTA, Christmas factor and AHF were performed as described before¹⁶ on substrates of plasma from patients with congenital clotting deficiencies. Fletcher factor was assayed by incubating a 0.1 ml test sample with 0.1 ml kaolin-Gliddex-P and Fletcher trait plasma in 10 × 75 mm glass tubes at 37°C for 1 min. 0.1 ml 0.025 M CaCl₂ was then added to the tubes and the clotting time was measured at 37°C. The results were expressed as the clotting time and as the percentage of activity relative to that in the mixture with normal rabbit γG.

the rabbits received a further injection of this kallikrein without adjuvant. Antiserum was collected 1 week thereafter. When subjected to immunodiffusion in 0.9% Agarose, the unabsorbed antiserum gave three precipitin lines with normal, Hageman trait or plasma thromboplastin antecedent (PTA)-deficient plasma, but only two lines with Fletcher trait plasma. After absorption with Fletcher trait plasma, the antiserum was adsorbed with tricalcium phosphate, subjected to 60°C for 60 min to inactivate clotting factors, and the γ-globulin fraction was separated by treatment with *n*-octanoic acid and ammonium sulphate¹². As a control, normal rabbit γ-globulin was prepared in the same way. The γ-globulin prepared from absorbed anti-kallikrein serum (anti-kallikrein antibody) formed a single precipitin line of identity against normal, Hageman trait or PTA-deficient plasma, and purified kallikrein or prekallikrein, whereas it formed no recognisable line against Fletcher trait plasma. The absorbed anti-kallikrein antibody retained the ability of unabsorbed antiserum to neutralise the kinin-generating activity of purified plasma kallikrein.

Normal human pooled citrated plasma, prepared from twenty-five normal adult males, was incubated with equal amounts of either normal rabbit γ-globulin or anti-kallikrein antibody at 37°C for 60 min. The mixtures were then tested in screening tests of blood coagulation. The thrombin time, prothrombin time and Russell's viper venom time were the same in both mixtures (Table 1). In contrast, the kaolin-activated partial thromboplastin time (kaolin PTT) was prolonged considerably in a mixture containing anti-kallikrein antibody (298.4 s) compared with that of a mixture containing normal rabbit γ-globulin (67.0 s).

These experiments suggested that the anti-kallikrein anti-

body interfered with an early phase of the intrinsic pathway. Four clotting factors have been recognised as participants of this stage: Hageman factor (factor XII), PTA (factor XI), Christmas factor (factor IX) and antihaemophilic factor (AHF, factor VIII). The functional activity of each of these clotting factors, assayed after incubation of normal plasma with anti-kallikrein antibody at 37°C for 60 min, was unaltered, as though the prolonged kaolin PTT was not due to the neutralisation of these clotting factors (Table 2). The Fletcher factor activity of normal plasma, assayed on the substrate of Fletcher trait plasma, however, was reduced sharply by incubation with as little as a four-fold dilution of anti-kallikrein antibody (less than 1% activity compared with a control). These experiments demonstrated that the incubation of normal plasma with anti-kallikrein antibody resulted in the impaired clotting through the intrinsic pathway by depleting its Fletcher factor activity.

The use of anti-kallikrein antibody in this study further supports the view that a plasma kallikrein may be required for the *in vitro* intrinsic pathway of blood coagulation. The exact site at which kallikrein participates in the intrinsic pathway is not yet settled, but evidence has been obtained that kallikrein may be required for the action of the activated Hageman factor¹³. Recently, Gjønnæss presented data suggesting that under certain conditions the extrinsic pathway of blood clotting may also be activated through an action of a plasma kallikrein on factor VII¹⁴. These studies serve once again to emphasize the intimate relationships between different systems of host-defense mechanisms, here the clotting and the kallikrein-kinin system.

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- Davie, E. W., and Ratnoff, O. D., *Science*, **145**, 1310 (1964).
- MacFarlane, R. G., *Nature*, **202**, 498 (1964).
- Bennett, B., and Ratnoff, O. D., *The Medical Clinics of North America*, **56**, 95 (1972).
- Hathaway, W. E., Belhasen, L. P., and Hathaway, H. S., *Blood*, **26**, 521 (1965).
- Hattersley, P. G., and Hayse, D., *Br. J. Haemat.*, **18**, 411 (1970).
- Wuepper, K. D., *Inflammation, Mechanisms and Control* (edit. by Lepow, I. H., and Ward, P. A.), 93 (Academic Press, New York, 1972).
- Saito, H., Donaldson, V. H., and Ratnoff, O. D., *J. clin. Invest.*, **52**, 72a (1973).
- Weiss, A. S., Gallin, J. I., and Kaplan, A. P., *Fed. Proc.*, **32**, 845 (1973).
- Saito, H., Ratnoff, O. D., Marshall, J. S., and Pensky, J., *J. clin. Invest.*, **52**, 850 (1973).
- Siegelman, A. M., Carlson, A. S., and Robertson, T., *Arch. Biochem. Biophys.*, **97**, 159 (1962).
- deJalon, P. G., Bayo Bayo, M. M., and deJalon, M. G., *Farmacoterapia Actual Madrid*, **2**, 313 (1945).
- Steinbuch, M., and Audran, R., *Arch. Biochem. Biophys.*, **134**, 279 (1969).
- Saito, H., Ratnoff, O. D., and Donaldson, V. H., *Circulation Res.*, (in the press).
- Gjønnæss, H., *Thrombos. Diathes. Haemorrh.*, **28**, 169 (1972).
- Forman, W. B., Ratnoff, O. D., and Boyer, M. M., *J. Lab. clin. Med.*, **72**, 455 (1968).
- Ratnoff, O. D., and Davie, E. W., *Biochemistry*, **1**, 967 (1962).

Lectin purification using formalinised erythrocytes as a general affinity adsorbant

MANY carbohydrate-binding proteins, called lectins, purified from plant extracts¹, are proving useful for the study of protein-carbohydrate interactions, cell-surface-initiated differentiation of cells and the topography of cell surface constituents^{1,2}. A general method for purification of lectins would markedly augment this work. Affinity chromatography has been successful for purification of some lectins² although others require conventional chemical fractionation. Purification may be simplified by the availability of commercial carbohydrate polymer adsorbants: for example Sephadex G-50³ for concanavalin A and Sepharose 4B for the carbohydrate-binding protein from *Dictyostelium discoideum*⁴.

Affinity chromatography can be difficult, however, if adsorbants must be manufactured. Purification of lectins would be easier if a general class of natural insoluble affinity adsorbants were available. Since lectins are generally identified by the ability to agglutinate erythrocytes of an appropriate type, we have tried formalinised erythrocytes as the affinity adsorbant. They have the advantage of being stable, not lysing in hypotonic or hypertonic media or when exposed to high concentrations of lectins. We found that formalinised erythrocytes bind lectins efficiently and release them on addition of appropriate sugars. Our method makes possible preparation of highly purified lectins with a minimum of effort and should prove applicable to all proteins which bind to carbohydrates on cell surfaces.

Crude plant seed extracts (Table 1) were assayed for the ability to agglutinate several types of formalinised erythrocytes⁵, and the type which gave the highest agglutination titre was used for purification. Assays were performed in microtitre V plates (Cooke Engineering) using serial two-fold dilutions of the extracts. Each well contained 25 μ l of lectin solution in 0.15 M NaCl (buffered to pH 6.4 for assays of concanavalin A) and 25 μ l of a 2.5% (v/v) suspension of formalinised erythrocytes in 0.15 M NaCl. Patterns were read 45 min after starting the reaction. The affinity of the erythrocyte adsorbant for lectin was determined by adding various amounts of erythrocytes to crude extract, shaking for 10 min and assaying the adsorbed supernatant for residual lectin activity after centrifugation at 10,000g for 10 min. For all lectins a ratio of erythrocytes to crude extract was chosen such that at least 95% of agglutination activity was adsorbed.

For purification the predetermined amounts of extract and formalinised erythrocytes were combined, and 0.15 M NaCl

was added to bring the final volume to a 30% (v/v) cell suspension, which was incubated at room temperature for 30 min with gentle shaking. The cells were then washed four times with 10 cell volumes of saline with centrifugation at 3,000g for 5 min. The lectin was eluted by shaking the washed cells (30% v/v) in 0.15 M NaCl containing 0.3 M sugar. After 30 min the supernatant was collected by centrifugation at 5,000g for 5 min, recentrifuged at 10,000g for 20 min and then exhaustively dialysed against 0.15 M NaCl to remove the sugar. The formalinised erythrocytes could be washed and used for another purification.

By this procedure, we obtained highly purified lectins in high yield from four plant sources (Table 1). Crude extracts containing concanavalin A and wheat germ agglutinin (WGA), were purified partially before adsorption to erythrocytes, whereas *Ulex* and lima bean lectins were purified directly from crude extracts. The binding capacity of the formalinised erythrocytes varied but was always sufficiently high for efficient purification. Under the conditions used binding was in the range of 0.4–1.7 mg lectin per packed ml of erythrocyte adsorbant and recoveries ranged from 50 to 100%. In all cases substantial quantities of pure lectin could be obtained with small amounts of adsorbant, so that conventional centrifugation facilities are adequate for preparative procedures several orders of magnitude larger than reported here.

The purity of the lectins was determined by polyacrylamide gel electrophoresis in a sodium dodecyl sulphate (SDS) system (Fig. 1). With concanavalin A, WGA and lima bean agglutinin a single protein band was observed after staining. With *Ulex* we found approximately equal amounts of two closely migrating proteins. All molecular weights agreed with previous determinations—WGA, 23,000⁶; lima bean, 30,000⁷; concanavalin A, 25,000⁸. With *Ulex*, the two closely migrating bands have molecular weights of 43,000 and 45,000. The subunit molecular weights of this lectin have not been reported previously, but the molecular weight of the intact molecule is approximately 160,000⁹. Thus, the *Ulex* lectin may be a tetramer. Commercially available WGA and concanavalin A (Miles) were identical by SDS gel analysis with our purified products. The agglutination activity per mg protein of these commercial lectins was equal to or slightly less than those prepared in our laboratory.

These findings suggest that formalinised erythrocytes can be used as a general affinity adsorbant for the purification of lectins from many sources. The simplicity of the method and the high yields and purity of the product recommend it for general use.

TABLE 1 Purification of four lectins by adsorption to formalinised erythrocytes

Source	mg Crude protein added to suspension	FE type	FE (packed ml)	Eluant	mg Recovered	% Lectin recovered*
Jack bean meal	9	Chicken	3	α -Methyl-D-mannopyranoside	1.7	57
Wheat germ lipase	84	Human Type A ₁	3	N-Acetyl-D-glucosamine	2.9	60
<i>Ulex europaeus</i> seeds	64	Human Type O	3	L-Fucose	1.8	50
Lima beans (<i>Carolina sieva</i>)	300	Human Type A ₁	5	N-Acetyl-D-galactosamine	2.0	100

FE, formalinised erythrocytes. Crude extracts from jack bean meal (Sigma) and wheat germ lipase (Miles) were partially purified according to published procedures¹¹, whereas crude extracts of *Ulex europaeus* seeds (F. W. Schumacher Co., Sandwich, Massachusetts), and lima beans (W. Atlee Burpee Co., Clinton, Iowa) were used directly. The jack bean meal was extracted in 0.15 M NaCl. The extract was concentrated with (NH₄)₂SO₄ and dialysed against 0.15 M NaCl. The product was adsorbed to formalinised erythrocytes. The wheat germ lipase was suspended in water, heated for 10 min at 58°C, chilled to 4°C, centrifuged at 10,000g for 15 min and the supernatant filtered, concentrated by ultrafiltration and made up to final concentration of 0.25 M NaCl, 0.05 M sodium phosphate, pH 7.0 before formalinised erythrocytes were added for adsorption. *Ulex europaeus* seeds and lima beans (100 g of each) were ground in a coffee grinder and extracted in 1.5 l 0.15 M NaCl for 15 h at 4°C with gentle shaking. The resulting suspension was filtered through cheesecloth, centrifuged at 10,000g for 30 min, concentrated by ultrafiltration and adsorbed with formalinised erythrocytes.

* % of lectin activity present in the initial extract which was recovered in the final product.

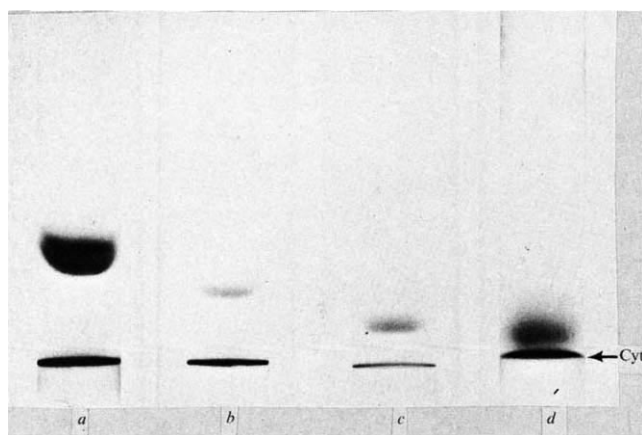


FIG. 1 Polyacrylamide gel electrophoresis of purified lectins. Lectins (100 to 300 μ g) were dissolved in 0.1% SDS, 0.01 M β -mercaptoethanol, applied to 10 cm polyacrylamide gels (2.5% stacking gel, 7.5% running gel) and electrophoresed in 0.1% SDS, 25 mM Tris, 192 mM glycine, pH 8.4. The gels were stained with Coomassie blue¹² and molecular weights estimated by comparison with protein standards¹³. Cytochrome *c* (Cyt) was added to all samples. *a*, *Ulex* agglutinin; *b*, lima bean agglutinin; *c*, concanavalin A; *d*, wheat germ agglutinin.

In a search of the literature for a method which might be similar to ours we found that Avrameas and Guilbert¹⁰ used glutaraldehyde-fixed erythrocyte stromata as affinity adsorbants for lectins. Since neither the binding capacity of this adsorbant for lectins nor the electrophoretic purity of the products are described, it is difficult to compare their method with that described here.

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- ¹ Sharon, N., and Lis, H., *Science*, **177**, 949 (1972).
- ² Lis, H., and Sharon, N., *A. Rev. Biochem.*, **42**, 541 (1973).
- ³ Agrawal, B. B. L., and Goldstein, I. J., *Biochim. biophys. Acta*, **147**, 262 (1967).
- ⁴ Rosen, S. D., Kafka, J. A., Simpson, D. L., and Baronides, S. H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2554 (1973).
- ⁵ Butler, W. T., *J. Immun.*, **90**, 663 (1963).
- ⁶ LeVine, D., Kaplan, M. J., and Greenaway, P. J., *Biochem. J.*, **129**, 847 (1972).
- ⁷ Gould, N. R., and Scheinberg, S. L., *Archs biochem. Biophys.*, **137**, 1 (1970).
- ⁸ Wang, J. L., Cunningham, B. A., and Edelman, G. M., *Proc. natn. Acad. Sci. U.S.A.*, **168**, 1130 (1971).
- ⁹ Matsumoto, I., and Osawa, T., *Biochem. biophys. Acta*, **194**, 180 (1969).
- ¹⁰ Avrameas, S., and Guilbert, B., *Biochimie, Paris*, **53**, 603 (1971).
- ¹¹ Ginsberg, V. (ed.) *Methods in Enzymology*, **27** (Academic Press, New York and London, 1972).
- ¹² Fairbanks, G., Steck, T. L., and Wallach, D. F. H., *Biochemistry*, **13**, 2606 (1971).
- ¹³ Shapiro, A. L., Vinuela, E., and Maizel, jun., J. V., *Biochem. biophys. Res. Commun.*, **28**, 815 (1967).

Autoradiography of intravenously injected ¹⁴C-nicotine indicates long-term retention in the respiratory tract.

INVESTIGATIONS using autoradiography¹ to monitor the distribution in mice of ¹⁴C-labelled nicotine administered intravenously showed a consistently high concentration in the entire respiratory tract. A number of mice, some of them pregnant, were each given a single intravenous dose and then were autoradiographed at postinjection times varying between 5 min and 30 d. The injected dose was 0.4 μ Ci per g body weight (corresponding to 1.79 μ g nicotine per g body weight) for the non-pregnant mice, and 0.25 μ Ci per g body

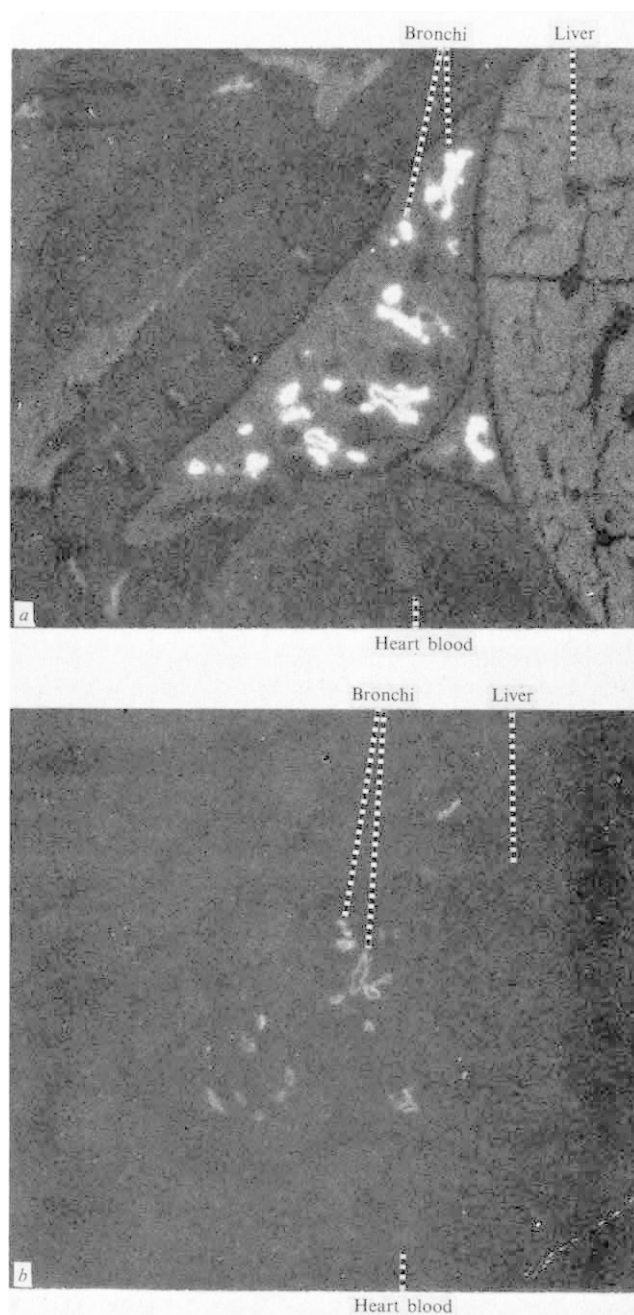


FIG. 1 Details of autoradiograms of mice, *a*, 24 h and, *b*, 30 d after intravenous injection of ¹⁴C-nicotine. Note the marked accumulation and retention in the bronchi (white areas).

weight (corresponding to 1.12 μg nicotine per g body weight) for the pregnant mice. The nicotine used was labelled in the methyl group (The Radiochemical Centre, Amersham, England, specific activity 73 μCi per mg^{-1}). Autoradiograms of whole body sagittal sections of the mice were made as described earlier¹. The times of exposure varied from 2 to 4 weeks.

At 5 min there were many sites of localisation in addition to the respiratory tract, such as the central nervous system (CNS), ganglia, and the adrenal glands which have previously been reported by our coworkers^{2,3}. After 1 h and later, the respiratory tract was increasingly dominant in the distribution picture as the radioactivity disappeared from the other areas. The main site of accumulation seemed to be the respiratory mucosal lining: the nasal mucosa, larynx, trachea and bronchi (Fig. 1a). The same pattern persisted after 30 d, although the concentration in the bronchi had now decreased it was still significant (Fig. 1b).

Most other tissues were rather rapidly cleared of the radioactive substance. After 30 d, however, there was still a low level present in the skeletal muscles and in the myocardium. Radioactive material was also observable in the urinary bladder. Although less persistent, a high uptake and retention could also be seen in the oesophageal mucosa.

In the pigmented animals a strong and persistent accumulation occurred in the tissues containing melanin. This has earlier been observed for many polycyclic drugs and seems (providing the dose exceeds a certain critical level) to cause damage of the tissues involved, for example, the skin, eye, inner ear and substantia nigra of the brain^{4,5}.

Long term accumulation in the respiratory tract, however, has not been previously observed in our autoradiographic studies of various types of chemicals. In full term foetuses, a selective accumulation could be seen in the respiratory tract (larynx, trachea and bronchi) and in the melanin bearing tissues.

Further investigations are needed to establish the chemical structure of the labelled compound which accumulates in the respiratory tract. To judge from the rapidity with which the uptake proceeds, it seems likely that the nicotine is trapped as such, but may later be metabolised. The mechanism of accumulation also needs further investigation. Affinity for some endogenous substance in the respiratory mucosal cells is one possibility.

If the observed accumulatory mechanism is also present in humans, smoking may lead to a considerable retention of some nicotine derivative in the respiratory mucosa. Oddly enough, an accumulation should also be expected if nicotine is administered by other routes than by inhalation (such as the use of snuff, chewing tobacco and nicotine tablets). It might, therefore, be worthwhile to look into the possible role of this accumulatory mechanism with respect to the pathogenesis of respiratory cancer and of bronchitis.

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Effect of thiocyanate on nitrosation of amines

CARCINOGENIC nitrosamines are formed by chemical reaction between nitrous acid and secondary and some tertiary amines in the stomachs of rodents¹⁻³. Nitrosamines could be formed similarly in the human stomach and so present a carcinogenic hazard to man. The nitrosation reaction is catalysed by some anions⁴ particularly thiocyanate^{5,6}. Thiocyanate is secreted in saliva which in non-smokers normally contains about 50 mg l^{-1} (approximately 1 mM) but in smokers contains three to four times this concentration⁷. In the absence of catalyst the nitrosation of N-methylaniline and some other secondary amines is maximal at pH 3, but in the presence of thiocyanate the reaction proceeds much more rapidly in acid conditions, such as in gastric juice, between pH 1 and 2 (Fig. 1). At pH 1.5, 1.0 mM thiocyanate increases the rate of reaction about 550 times. Under these acid conditions below pH 2 the nitrosation reaction rate is proportional to the concentrations of (1), nitrite; (2), thiocyanate; and (3), amine⁸. At pH 3.5 and greater thiocyanate has less catalytic action and the predominant reaction is proportional to the square of the nitrite concentration, but is slow.

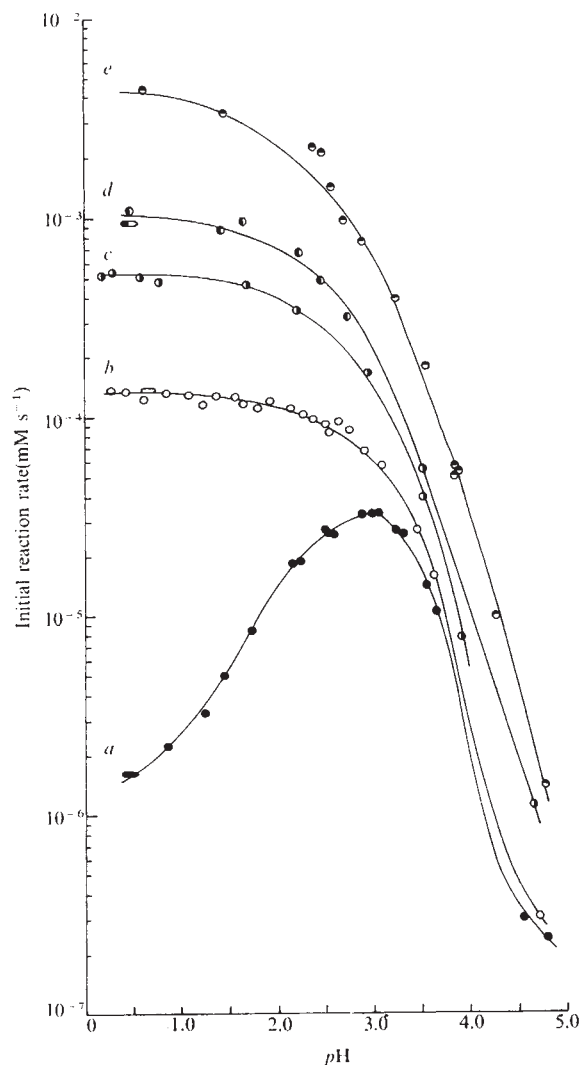


Fig. 1 Initial rate of reaction of N-methylaniline (0.1 mM) and sodium nitrite (0.1 mM) with various concentrations of sodium thiocyanate against pH at 25° C. a, thiocyanate absent; b, 0.025 mM thiocyanate; c, 0.1 mM thiocyanate; d, 0.25 mM thiocyanate; e, 1.0 mM thiocyanate.

¹ Ullberg, S., *Acta Radiol.*, supplement 118 (1954).

² Hansson, E., and Schmitterlöv, C. G., *J. Pharmac. exp. Ther.*, **137**, 91 (1962).

³ Schmitterlöv, C. G., and Hansson, E., *Nature*, **194**, 298 (1962).

⁴ Lindquist, N. G., and Ullberg, S., *Acta Pharmac. Toxicol.*, **31**, supplement 2 (1972).

⁵ Lindquist, N. G., *Acta Radiol.*, supplement 325 (1973).

The volume of saliva secreted by man is about 1.5 l d^{-1} and that of gastric juice between 2 and 3 l d^{-1} . If the daily intake of water in food and drink were 3 l d^{-1} then saliva would be diluted some five-fold in the stomach. The thiocyanate concentration in the stomach could therefore be about 0.2 mM in non-smokers and 0.6 mM in smokers; these concentrations of thiocyanate would increase the nitrosation reaction 100 and 300 times at $\text{pH } 2$. Saliva also contains up to 200 mg l^{-1} nitrate^{9,10} which is probably derived from food, water and tobacco smoke. Some of the nitrate is reduced to nitrite in the mouth. The smoke from a cigarette contains oxides of nitrogen which might produce 0.5 mg of nitrite in the body so that 20 cigarettes could yield 10 mg nitrite. This amount of nitrite could also be present in 200 g of ham or Frankfurter sausages.

Cigarette smoke contains cyanide compounds which are converted to thiocyanate in the body by the action of rhodanese (thiosulphate-sulphur transferase). The smoke from a cigarette contain up to 0.5 mg cyanides which could yield double this amount of thiocyanate. The daily output of saliva of smokers, however, contains some 200 mg more thiocyanate than that of non-smokers. Thiocyanate is secreted both by the kidney and salivary glands and the volumes of urine and saliva are about the same. Smoking generally increases the flow of saliva¹¹.

Non-smokers excrete three times more thiocyanate in urine than in saliva, that which is secreted in saliva being reabsorbed from the alimentary tract. In smokers the urinary and salivary thiocyanate excretions are about equal. Thus smoking seems to change the route of thiocyanate excretion either by inhibition of kidney secretion or stimulation of salivary secretion. Although cigarette smoke inhibits the activity of rhodanese of guinea pig tissues it increases the thiocyanate concentration of the salivary glands, lung, kidney and brain¹². This is because rhodanese activities are high and smoke contains cyanide compounds.

Nitrosamines are formed in cigarette smoke by the reaction of oxides of nitrogen with secondary and tertiary amines. The most abundant nitrosamine in cigarette smoke seems to be nitrosornicotine¹³ which is carcinogenic¹⁴ and formed from nicotine. The formation of nitrosornicotine would be greater in the more acid smoke of British cigarettes than in the less acid smoke of other cigarettes, cigars or pipes. The synthesis of nitrosamines in the stomach is probably greater in smokers than non-smokers because of the hydrocyanic acid and oxides of nitrogen present in tobacco smoke. Reduction in the concentration of oxides of nitrogen in tobacco smoke should reduce the amount of nitrosamines formed by the smoke itself and in the stomach.

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- ¹ Sander, J., and Bürkle, G., *Z. Krebsforsch.*, **75**, 301 (1969).
- ² Mirvish, S. S., Greenblatt, M., and Kommineni, U. R. C., *J. natn. Cancer Inst.*, **48**, 1311 (1972).
- ³ Lijinsky, W., Taylor, H. W., Snyder, C., and Nettesheim, P., *Nature*, **244**, 176 (1973).
- ⁴ Ridd, J. H., *Q. Rev.*, **15**, 418 (1961).
- ⁵ Boyland, E., Nice, E., and Williams, K., *Food Cosmet. Toxicol.*, **9**, 639 (1971).
- ⁶ Fan, T.-Y., and Tannenbaum, S. R., *J. agric. Fd Chem.*, **21**, 14 (1973).
- ⁷ Densen, P. M., Davidow, B., Bass, H. E., and Jones, E. W., *Archs envir. Hlth*, **14**, 865 (1967).

- ⁸ Boyland, E., and Walker, S. A., *Nitroso Compounds Analysis and Formation* (International Agency for Research on Cancer, Lyon, in the press).
- ⁹ Schönbein, *Jber. chem.* p. 98 (1862).
- ¹⁰ Ville, J., and Mestrezat, W., *C. r. Séanc. Soc. Biol.*, **63**, 231 (1907).
- ¹¹ Barylko-Pikielna, N., and Panghorn, R. M., *Archs envir. Hlth*, **17**, 739 (1968).
- ¹² Schievelbein, H., Werle, E., Schulz, E. K., and Banmeister, R., *Arch. Pharmak exp. Path.*, **262**, 358 (1969).
- ¹³ Hoffman, D., Rathcamp, G., and Liu, Y. Y., *Nitroso Compounds Analysis and Formation* (International Agency for Research on Cancer, Lyon, in the press).
- ¹⁴ Boyland, E., Roe, F. J. C., and Gorrod, J. W., *Nature*, **202**, 1126 (1964).

Thymidylate concentration in megaloblastic anaemia

MEGALOBlastic anaemia is thought to be caused by a disturbance of DNA synthesis in the bone marrow. The most common causes are deficiencies of folate or vitamin B_{12} which are thought to interfere directly or indirectly with supply of thymidine triphosphate (dTTP, thymidylate) one of the four immediate precursors of DNA^{1,2} (Fig. 1). We have previously shown that acute folate deficiency caused by the antifolate drug, methotrexate, does indeed cause a fall in the intracellular concentration of dTTP³. The present paper reports studies of the cellular concentration of dTTP and of the other three deoxyribonucleotide precursors of DNA, deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP) and deoxycytidine triphosphate (dCTP) in the bone marrow cells and phytohaemagglutinin (PHA)-stimulated lymphocytes from patients with untreated megaloblastic anaemia due to vitamin B_{12} or folate deficiency. Surprisingly, no reduction in dTTP concentration was found in either cell.

Bone marrow was obtained from 20 patients with untreated megaloblastic anaemia (16 Addisonian pernicious anaemia, 4 nutritional folate deficiency). Their haemoglobin levels ranged from 3.1 to 9.8 g per 100 ml and all had florid megaloblastic marrow changes and adequate iron stores. Peripheral lymphocytes were obtained from 15 of these patients. Normal control subjects consisted of 70 healthy adult members of the medical and technical staff of the Hammersmith Hospital. Bone marrow samples were obtained from 13 of these normal subjects and from 14 adult patients with untreated acute myeloid leukaemia in relapse (30–90% blast cells in the marrow).

The deoxyribonucleotide concentrations in bone marrow are shown in Table 1. The levels of all four, dATP, dGTP,

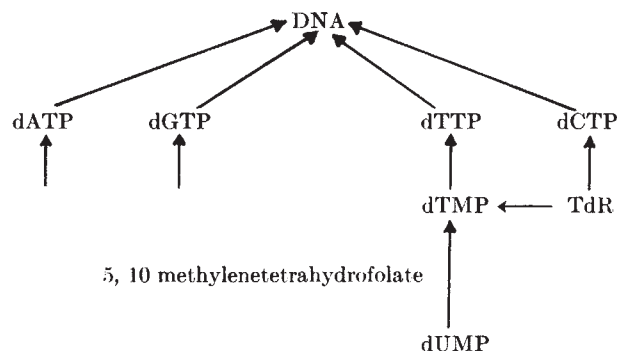


FIG. 1 The synthesis of DNA from four deoxyribonucleoside triphosphates (d, deoxy; A, adenosine; G, guanosine; T, thymidine; C, cytidine; U, uridine; TP, triphosphate; MP, monophosphate; TdR, thymidine).

TABLE 1 Deoxyribonucleoside triphosphate pools in human marrow cells

Diagnosis	dATP			dGTP			dTTP			dCTP		
	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range
Normal	13	1.5	0.2-3.0	10	0.4	0.1-0.6	14	1.4	0.5-2.2	14	0.6	0.1-1.0
Acute myeloblastic leukaemia	14	3.1	0.2-9.3	14	1.5	0.3-4.9	18	5.8	0.6-22	15	1.2	0.3-4.9
Megaloblastic anaemia	17	3.3	0.7-13.6	11	2.2	0.1-5.7	20	5.4	0.8-23.7	9	2.0	0.3-4.6

Bone marrow cells were collected into 5 ml ice-cold Hanks' balanced salt solution containing 500 units sodium heparin. After centrifugation, mature red cells were haemolysed in distilled water for 30 s before restoration of isotonicity with 0.6 M KCl. The cells were washed once in ice-cold phosphate-buffered saline. Extraction of the deoxyribonucleotides was performed by adding 1 ml 60% methanol, cooled to -20°C , to the cell pellet⁴. The tubes were then stood overnight at 4°C before removal of the methanol by evaporation. The assay of the deoxyribonucleotides has been described^{5,6}. For estimation of dATP and dTTP, poly(dA-dT) was used as primer while for dGTP and dCTP, DNA was used as primer. Radiochemicals were purchased from Radiochemical Centre, Amersham, poly(dA-dT) and DNA polymerase (*Micrococcus luteus*) from Miles Laboratories and calf thymus DNA from Sigma Laboratories.

Results are expressed as pmol per 10^6 cells.

dTTP, and dCTP, were greater in megaloblastic than normal marrow cells. It is known, however, that proliferating cells, in general, show greater deoxyribonucleotide concentrations than resting cells⁷. Since megaloblastic marrows contain a greater proportion of primitive cells than normal, the levels of the DNA precursors are also compared with those in the bone marrows of patients with untreated myeloblastic leukaemia which also contain a high proportion of primitive (albeit abnormal) cells. There was a wide variation in individual results, but there was no significant difference between the mean dTTP concentration in the marrow cells of patients with untreated megaloblastic anaemia and acute myeloblastic leukaemia. The ratio of dTTP concentration to that of dATP, dGTP or dCTP, was also similar in these two diseases.

The mean deoxyribonucleotide concentrations in PHA-stimulated lymphocytes from patients with megaloblastic anaemia were not significantly different from normal at either 48 h or 72 h of culture (Table 2). In particular, the dTTP concentrations were similar in megaloblastic and normal lymphocytes. In neither bone marrow nor peripheral lymphocytes were the deoxyribonucleotide concentrations different between the folate-deficient and vitamin B₁₂-deficient megaloblastic cells (not shown).

These results show that the free concentrations of the immediate precursors of DNA, the four deoxyribonucleoside triphosphates, are normal in the PHA-transformed lymphocytes of patients with untreated megaloblastic anaemia. The concentrations of these four precursors are increased in megaloblastic bone marrow compared with normal bone marrow, but only in keeping with the increased proportion of primitive cells in megaloblastic marrow compared with normal. No selective reduction of thymidine triphosphate (dTTP) concentration could be demonstrated in either cell system as might have been expected both on the basis of previous observations of a block in thymidylate synthesis in bone marrow and in PHA-stimulated lymphocytes of patients with untreated megaloblastic anaemia^{1,2} and on the basis of more recent observations of a dramatic decrease in cell dTTP concentration produced by methotrexate *in vitro*³.

Various theories may be proposed to explain the rather surprising finding of a normal cell thymidylate concentration

in megaloblastic anaemia. It seems most likely to us that compensation has occurred in the cells of patients with chronic folate or vitamin B₁₂ deficiency to maintain normal cell dTTP concentrations despite defective synthesis of thymidylate from deoxyuridylate (Fig. 1). This compensation could take the form of increased salvage of preformed thymidine from degradation of DNA in the bone marrow and in other tissues. The activity of the enzyme, thymidine kinase, which is necessary for the salvage of preformed thymidine is indeed increased in the bone marrow cells and PHA-transformed lymphocytes in untreated megaloblastic anaemia^{9,10}. Moreover, intramedullary haemolysis would lead to excessive release of preformed thymidine in the marrow in megaloblastic anaemia. Slow DNA synthesis, known to be a feature of megaloblasts¹¹, could also contribute to maintenance of the dTTP concentration by reducing utilisation of dTTP.

Some other explanations for the normal dTTP findings are possible. It could be that the present finding of normal dTTP concentrations in megaloblastic anaemia is artefactual as a result of replenishment of the dTTP concentration *in vitro* in our cultures but this is unlikely. Though the medium, TC 199, used for culture of lymphocytes does contain thymine, 10^{-6} M, culture in a specially prepared thymine-free TC 199 made no difference to the measured dTTP concentration in PHA-stimulated megaloblastic lymphocytes from three patients (unpublished observations). Further, the bone marrow dTTP estimations were made on marrow samples cooled in Hanks' solution (free of thymine or thymidine) and extracted immediately after aspiration. Another potential source of artefact is that a compound which accumulates in megaloblastic cells other than dTTP might be measured as dTTP by the technique used here. We have no evidence to support or refute this possibility, though in view of our finding of a consistent decrease in measured dTTP concentration with methotrexate *in vitro*³, we feel it is unlikely. A further possibility is that an overall normal cellular dTTP concentration in megaloblastic anaemia may mask an intracellular redistribution of this compound with a reduction in nuclear dTTP masked by a increase in the extranuclear concentration of this compound. Hydroxyurea, which inhibits DNA synthesis mainly by antagonism of ribonucleotide reductase, has also been suggested to damage the nuclear mem-

TABLE 2 Deoxyribonucleoside triphosphate concentrations in human PHA-stimulated lymphocytes

Cells	Time of culture	dATP			dGTP			dTTP			dCTP		
		No.	Range	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
Normal	48 h	10	1.9-6.1	3.6	2	0.6-2.4	1.5	11	3.2-13.4	8.7	2	1.0-3.1	2.0
Megaloblastic anaemia	48 h	5	1.2-5.7	2.7	—	—	—	5	3.7-11.4	7.6	—	—	—
Normal	72 h	69	0.7-8.0	3.7	27	0.5-5.6	1.9	70	1.1-17.7	9.4	29	0.8-6.3	2.9
Megaloblastic anaemia	72 h	15	2.5-6.5	4.2	4	1.2-5.1	3.1	15	3.1-16.3	10.8	4	3.8-4.7	4.4

Lymphocyte cultures were set up in autologous serum and TC 199 as described⁸ except Triosil-Ficoll was used instead of iron filings to remove neutrophils. Cultures of 3×10^6 cells were made in 1 ml autologous serum and 2 ml TC 199 (Burroughs-Wellcome) and 0.1 ml PHA. After 48 or 72 h, the cells were washed twice in ice-cold phosphate-buffered saline.

Results are expressed as pmol per 10^6 cells

brane, and loss of deoxyribonucleotides from the nucleus may play a part in its action¹². Further studies of the sub-cellular localisation of dTTP in megaloblasts are, therefore, necessary. Finally, there has been discussion whether the mono or diphosphate rather than the triphosphate derivatives of the deoxyribonucleosides are the immediate DNA precursors. We have not measured the cell concentration of thymidine mono or diphosphate in megaloblastic anaemia but recent evidence does firmly support the deoxyribonucleoside triphosphates as the crucial precursors¹³.

In a previous study³ we found that acute folate deprivation caused by methotrexate *in vitro* in PHA-stimulated lymphocytes caused an increase in dATP concentration as well as a decrease in dTTP. The present results show that in chronic megaloblastic anaemia there is no increase in dATP but that the dATP concentration parallels that of the other deoxyribonucleotide triphosphates. Compensations must have occurred to balance the deoxyribonucleotide pools at a slower rate of DNA synthesis in the chronic state.

Whatever the explanation for the normal deoxyribonucleotide concentrations in untreated megaloblastic anaemia, our results provide further information on the disturbance of DNA synthesis in this disorder and complement previous observations that the base composition of the DNA itself is normal in the bone marrow in untreated megaloblastic anaemia¹⁴. They are consistent with the view that slowed DNA synthesis in some way gives rise to the characteristic morphology and intramedullary cell death of megaloblastic anaemia.

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- ¹ Metz, J., Kelly, A., Swett, V. C., Waxman, S., and Herbert, V., *Br. J. Haemat.*, **14**, 575 (1968).
- ² Das, K. C., and Hoffbrand, A. V., *Br. J. Haemat.*, **19**, 459 (1970).
- ³ Hoffbrand, A. V., and Tripp, E., *Br. med. J.*, **2**, 141 (1972).
- ⁴ Skoog, L., *Eur. J. Biochem.*, **17**, 202 (1970).
- ⁵ Solter, A. W., and Handschumacher, R. E., *Biochim biophys Acta*, **174**, 585 (1969).
- ⁶ Lindberg, U., and Skoog, L., *Analyt. Biochem.*, **34**, 152 (1970).
- ⁷ Soderhall, S., Larsson, A., and Skoog, L., *Eur. J. Biochem.*, **33**, 36 (1973).
- ⁸ Das, K. C., and Hoffbrand, A. V., *Br. J. Haemat.*, **19**, 203 (1970).
- ⁹ Bock, H. E., Hartje, J., Muller, D., and Wilmanns, W., *Klin. Wschr.*, **45**, 176 (1967).
- ¹⁰ Hoffbrand, A. V., Hooton, J., Lavoie, A., Tattersall, M. H. N., Ganeshaguru, K., and Tripp, E., *Eur. J. clin. Invest.*, **3**, 238 (1973).
- ¹¹ Wickramasinghe, S. N., Chalmers, D. G., and Cooper, E. H., *Nature*, **215**, 189 (1968).
- ¹² Adams, L. P., Berryman, S., and Thomson, A., *Biochim biophys Acta*, **240**, 455 (1971).
- ¹³ Friedland, A., *Nature new Biol.*, **243**, 105 (1973).
- ¹⁴ Hoffbrand, A. V., and Pegg, A. E., *Nature new Biol.*, **235**, 187 (1972).

Catecholamine transport through a lipid barrier

NORADRENALINE and adrenaline are stored in relatively high concentration together with nucleotides (mainly adenosine-5'-triphosphate (ATP)), bivalent cations (for example Ca^{2+} and Mg^{2+}) and water-soluble proteins, in special intracellular organelles such as chromaffin granules of adrenal medulla¹. In the uptake of catecholamines by these organelles a specific mechanism is probably involved at the level of the granular membrane². Furthermore, formation of low molecular weight complexes between catecholamines and ATP^{3,4} and mixed amine-nucleotide aggregates of apparent high molecular weight⁵⁻⁸ has been demonstrated. The present experiments deal with the role of these interactions for the uptake and storage of the amines. Evidence will be presented that an 'uphill' transport of neurotransmitters occurs if ATP is present on one side of an artificial lipid barrier and that this effect is connected with a reduction in the osmotic pressure of the biogenic monoamines by ATP.

Permeation studies were carried out at 37° C with commercially available equipment (resorption model of Stricker, SM 16750, Sartorius-Membranfilter GmbH, Göttingen), consisting of a diffusion cell with two compartments, A and B, of equal size (100 ml) separated by an artificial lipid barrier, and a peristaltic pump for the circulation of the aqueous solutions along the lipid barrier. The latter, which had an effective size of 80 cm² and a thickness of about 100 µm, was prepared by soaking a membrane filter (type RS, Sartorius, Göttingen) at 30° C with a mixture of 97.7% lauryl alcohol and 2.3% diethyl succinate. (–)-Noradrenaline (0.0095 mol l⁻¹) (purum, Fluka, Buchs) or 0.0055 mol l⁻¹ (–)-adrenaline (crystalline, Merck, Darmstadt) or 0.0083 mol l⁻¹ β-phenethylamine (Sigma, St Louis) were dissolved together with 0.024 mol l⁻¹ CaCl_2 in K–Na phosphate buffer of pH 5.8 (molarity: 0.067). ATP (0.10 mol l⁻¹) was added to the solution in compartment B, but not to A. The pH of the

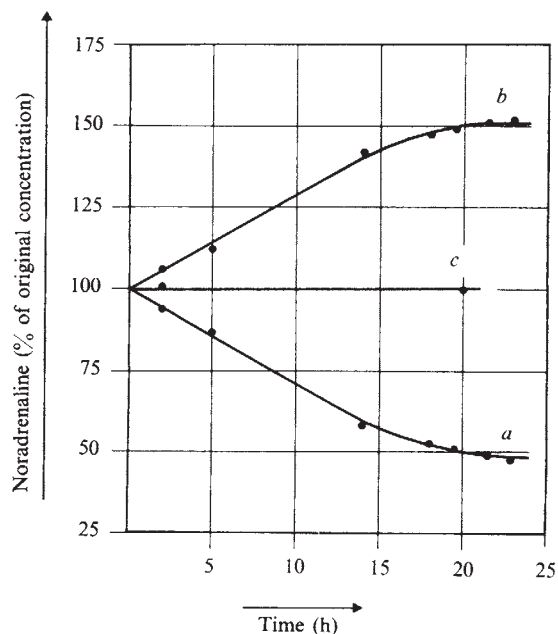


FIG. 1 Transport of noradrenaline through a lipid membrane from compartment A to compartment B at 37° C. Both compartments contain 0.0059 mol l⁻¹ noradrenaline at the beginning of the experiment. Compartment B also contains 0.10 mol l⁻¹ ATP. The pH in both compartments is adjusted to 5.8 and remains constant during the experiment. 100% Noradrenaline concentration at the beginning of the experiment; a, compartment A; b, compartment B; c, compartments A and B in the absence of ATP in compartment B. Results are the averages of four experiments.

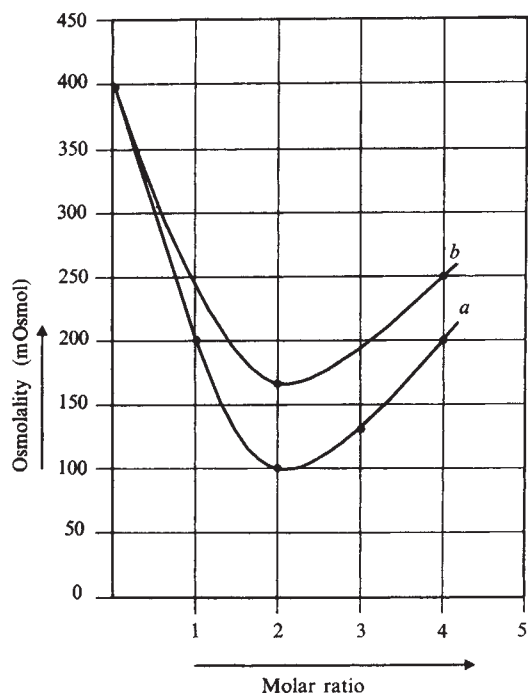


Fig. 2 Effect of added catecholamines on osmolality of an aqueous solution of 0.19 mol l^{-1} ATP, containing CaCl_2 in a molar ratio to ATP of 0.24 to 1, at 37°C . a, Catecholamine = noradrenaline; b, catecholamine = adrenaline. Results are average of three experiments.

solutions was readjusted to 5.8 with 2 N NaOH. Auto-oxidation of noradrenaline was prevented by adding 0.01% ascorbic acid (Hoffmann-La Roche, Basel) and by bubbling nitrogen through the solutions during the course of the experiments. The concentrations of noradrenaline⁹ and of ATP¹⁰ were checked by fluorescence spectroscopy, those of adrenaline and β -phenethylamine by ultraviolet spectroscopy. Analysis by fluorescence spectroscopy showed that the artificial lipid barrier was impermeable to ATP, corresponding in this respect to membranes of amine storage organelles¹¹.

Osmolalities were determined by isothermal distillation¹² at 37°C using aqueous NaCl as reference solutions. The osmolalities of all solutions were estimated by measurement of freezing point depression with a semi-micro osmometer (Knauer, Berlin). Controls of the osmotic pressure of the solutions of inorganic ions by isothermal distillation at 37°C showed that their osmolalities were practically independent of temperature. The osmotic pressure due to the inorganic ions was subtracted, so that the results presented in the graph corresponded to ATP (0.19 mol l^{-1}) and its mixtures with the catecholamines only. The pH of the solutions containing noradrenaline (0.76 mol l^{-1}) in a molar ratio to ATP of 4 to 1 was 5.8, that of the solutions of lower molar ratios or with ATP only correspondingly lower (0.6). Control experiments with adjustment of the pH of the ATP solution to 5.8 (using 2 N NaOH) in the absence of noradrenaline gave evidence that the pH effect on osmolality was negligible.

Figure 1 shows the 'uphill' transport of noradrenaline at 37°C . The amine content was the same in both compartments at the beginning of the experiment ($0.0059 \text{ mol l}^{-1}$), but compartment B also contained 0.10 mol l^{-1} ATP. During the experiment, the noradrenaline concentration in compartment A decreased markedly (curve a), and correspondingly an increase occurred in compartment B (curve b). Adrenaline behaved similarly to noradrenaline. No change in catecholamine concentration was to be seen in the absence of ATP from compartment B (curve c). Transport of noradrenaline into the ATP-containing compartment also occurred at higher

initial concentrations of the amine on both sides of the lipid barrier.

Figure 2 demonstrates the effect of the addition of noradrenaline and adrenaline on the osmolality of 0.19 mol l^{-1} aqueous solution of ATP at 37°C . The solutions also contained CaCl_2 in a molar ratio to ATP of 0.24 to 1. The results indicate that mixtures of ATP and noradrenaline and of ATP with adrenaline have substantially lower osmotic pressures than ATP alone. The fraction of catecholamines present in the dissociated state in these mixtures was about 10–20% (based on calculations of apparent average molecular weights obtained by isothermal distillation, sedimentation equilibrium and velocity experiments). In contrast, noradrenaline hydrochloride was dissociated to about 70–80% in the same range of concentrations (determined by isothermal distillation at 37°C). Minimum osmotic pressure existed at a molar ratio of 2:1, catecholamines to ATP, the osmotic pressure of ATP being reduced by a factor of 4 and 2.5 by the addition of noradrenaline and adrenaline respectively. On increasing the molar ratio to greater than 2:1, the osmotic pressure rose again, but even at a ratio 4:1 it was still lower than that of ATP alone.

The accumulation of catecholamines in the ATP-containing compartment B was probably due to intermolecular bonding between the amines and the nucleotide, which is confirmed by our osmolality measurements. Because of the interaction of catecholamines and ATP, the concentration of free catecholamines available for diffusion is likely to decrease in the ATP-containing compartment B, giving rise to a concentration gradient between compartments A and B of the free (diffusible) amine. The catecholamines would then penetrate by diffusion from compartment A into compartment B, where most of them are bound to ATP. The overall displacement of catecholamines from A into B probably stops when the concentration of the free catecholamines in the two compartments is the same. This view is supported by the finding that β -phenethylamine, which does not accumulate in the ATP-containing compartment of the diffusion chamber, did not display marked aggregation with ATP in the sedimentation equilibrium and velocity experiments (our unpublished observations).

The present results with an artificial lipid membrane show that in the presence of ATP and bivalent cations, catecholamines can be accumulated against a concentration gradient without a specific, energy-dependent transport mechanism being present. This finding confirms the previous hypothesis by which intragranular formation of aggregates between amines, nucleotides, bivalent cations and possibly proteins is an essential mechanism for the uptake and storage of biogenic amines by adrenal chromaffin granules and probably by other storage organelles, blood platelets for example¹³. A tentative structure for the intragranular organisation of catecholamines and ATP has recently been proposed¹⁴.

In conclusion, our experiments provide direct evidence that transport and accumulation of catecholamines can occur as a consequence of their aggregation with ATP. This aggregation may considerably increase the efficiency of the granular membrane pump in storing biogenic amines.

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¹ Smith, A. D., in *The Interaction of Drugs and Subcellular Components in Animal Cells*, 239 (Churchill, London, 1968).

² Taugner, G., *Naunyn-Schmiedeberg Arch. exp. Path. Pharmacol.*, **270**, 392 (1971).

- ³ Weiner, N., and Jandetzky, O., *Naunyn-Schmiedeberg Arch. exp. Path. Pharmacol.*, **248**, 308 (1964).
- ⁴ Pai, V. S., and Maynert, E. W., *Molec. Pharmacol.*, **8**, 82 (1972).
- ⁵ Berneis, K. H., Pletscher, A., and Da Prada, M., *Nature*, **224**, 281 (1969).
- ⁶ Berneis, K. H., Pletscher, A., and Da Prada, M., *Br. J. Pharmacol.*, **39**, 382 (1970).
- ⁷ Da Prada, M., Berneis, K. H., and Pletscher, A., *Life Sci.*, **10**(I), 639 (1971).
- ⁸ Berneis, K. H., Goetz, U., Da Prada, M., and Pletscher, A., *Naunyn-Schmiedeberg Arch. exp. Path. Pharmacol.*, **277**, 291 (1973).
- ⁹ von Euler, U. S., and Hamberg, U., *Acta physiol. scand.*, **19**, 74 (1949).
- ¹⁰ Holmsen, H., Holmsen, I., and Bernhardsen, A., *Analyt. Biochem.*, **17**, 456 (1966).
- ¹¹ Da Prada, M., and Pletscher, A., *Life Sci.*, **9**(II), 1271 (1970).
- ¹² Niederl, J. B., and Kasanof, D. R., *Science*, **100**, 228 (1944).
- ¹³ Berneis, K. H., Da Prada, M., and Pletscher, A., *Experientia*, **27**, 917 (1971).
- ¹⁴ Pletscher, A., Da Prada, M., Berneis, K. H., Steffen, H., Lütold, B., and Weder, H. G., in *Advances in Cytopharmacology*, **2** (Raven Press, in the press).

The contribution of activation processes to the length-tension relation of cardiac muscle

It is generally agreed that the form of the length-tension relation of a fully activated skeletal muscle is determined by the overlap of actin and myosin filaments within the sarcomere. Figure 1 shows the length-tension curve for tetanised frog skeletal muscle¹, which is thought to be fully activated at lengths above 75% L_{max} (ref. 2). This is the form of the curve that would be expected from any muscle of similar sarcomere structure to frog muscle, provided that it has a constant degree of activation (either complete or partial) at muscle lengths above 75% L_{max} . The sarcomere structure of cardiac muscle is similar to that of frog muscle³, yet the shape of its length-tension curve is strikingly different (Fig. 1). In skeletal muscle deviation from the 'ideal' curve shown in Fig. 1 has been attributed to variation in the degree of activation when the muscle is stimulated at different lengths. We have investigated this possibility in cardiac muscle by studying the effect on the length-tension

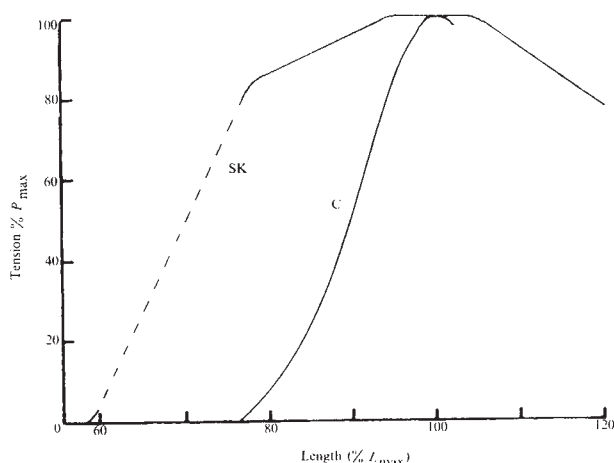


FIG. 1 The length-tension relationships for skeletal (Sk) and cardiac (C) muscle. The ordinate is the developed tension as a percentage of the maximum developed tension (P_{max}) and the abscissa is the length as a percentage of the length (L_{max}) at which developed tension is maximum. There is evidence that the sarcomere spacings at L_{max} are similar in frog skeletal and cat papillary muscle³. The dashed line on the skeletal muscle curve is the region over which activation may be incomplete².

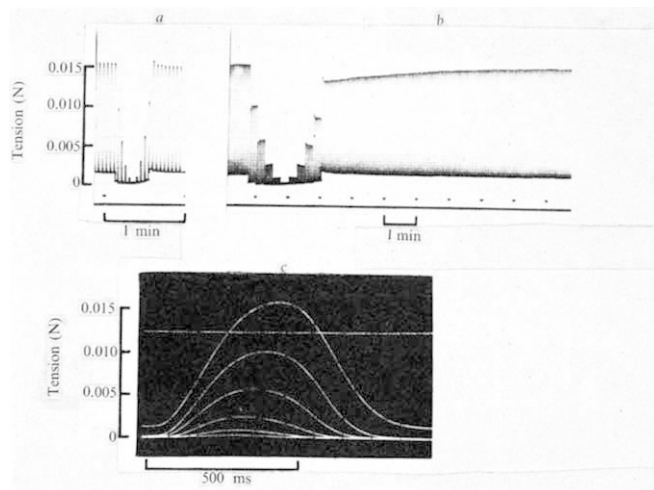


FIG. 2 Experimental records of twitch responses of a cat papillary muscle (L_{max} 3.3 mm, diameter 0.6 mm) to electrical stimulation at 20 min⁻¹. *a*, Experimental sequence used to obtain the length-tension curves shown in Fig. 3. The length of the muscle is first decreased and then increased again in five equal steps of 0.165 mm (5% L_{max}). Tension is measured in newtons. *b*, This shows an alternative sequence in which the muscle was held at each length for five beats: the difference between the first beat after a change of length and subsequent beats is attributed to the presence of a series viscoelastic element in the preparation. Note that tension production takes several minutes to recover to its previous steady level after the muscle has been returned to its original length. *c*, High speed (oscilloscope) records of the twitch responses during the descending part of the tracing shown in *a*.

relation of varying the degree of activation produced by electrical stimulation: we have done this by altering the concentration of calcium in the bathing medium and by using paired pulse stimulation⁴.

The experimental procedure was as follows. An isolated papillary muscle from the right ventricle of a kitten was stimulated electrically at 30° C to give isometric twitch contractions every 3 s (see ref. 5 for further details about technique). The length-tension curve was determined as quickly as possible by changing the length of the muscle between successive beats (Fig. 2a), starting near L_{max} and working down to a length at which no increase in tension was detectable on stimulation and then returning in similar steps almost to L_{max} . This procedure avoided the complications introduced by the slow changes in tension development that occur when the muscle is allowed to contract for a longer period at short lengths (compare Fig. 2b and a). The value of L_{max} was then estimated by stretching the muscle in smaller steps until the developed tension (peak tension minus resting tension) started to fall. The entire procedure was performed when the calcium concentration in the bathing solution was 2.0 mM, 0.5 mM, 8.0 mM and then repeated at 2.0 mM.

The resting tension and peak tension were measured at each length and the values obtained on the ascending and descending limbs of the cycle were averaged to minimise the influence of a viscous element or other factors⁵ which might make the first beat at lengths below L_{max} different from subsequent ones (see Fig. 2b). The results have been plotted in two ways, as in Fig. 3a and b, which show the data obtained from a representative muscle. In Fig. 3a the peak tension and resting tension are given in N mm⁻² as a function of the actual muscle (mm), and the estimated value of L_{max} is marked on each curve. The length-tension relation for the resting muscle is unaffected by changes in the calcium concentration, but the curve for peak tension is affected in several ways: there is a very slight shift of L_{max} to longer muscle lengths when the calcium concen-

tration is raised, but the main effect is a large potentiation of tension development, which is proportionally greatest at short muscle lengths. This is clearly seen in Fig. 3a, but it becomes much more obvious when the data are replotted so that the maximum tension developed at each calcium level is normalised to 100% and muscle lengths are expressed as a percentage of $L_{\max}$ (Fig. 3b). If the normalised curves are not superimposable at lengths other than $L_{\max}$, then the intervention must have altered the degree of activation relatively more at these lengths than at $L_{\max}$: the implication of this would be that activation processes in the muscle are length-dependent. Thus the conclusion from the results in Fig. 3b is that the form of the length-tension relation of cardiac muscle depends at least in part on a variation in the degree of activation with length. The 2 mM- Ca^{2+} data shown in Fig. 3 (and comparable data from three similar experiments) suggest that the form of the length-tension relation changes slightly with time, but any such effect is too small to affect our conclusion.

Since the 8.0 mM curve is shifted towards that expected from the sliding filament theory, one wonders if by increasing the activation further a curve which approximated more closely to the sliding filament theory could be obtained. To this end we have increased the degree of activation by paired pulsing which in some circumstances may produce tensions close to the maximum of which the muscle is capable at that length⁴. This altered the shape of the length-tension relation, but only to approximately the same extent as 8.0 mM calcium.

One possible objection to our results is that peak tension is not a satisfactory index of contractility if the time to peak tension is altered by the intervention being investigated (see Fig. 2c)⁶. However, the length-tension relation for $(dP/dt)_{\max}$, an index of contractility that is unaffected by changes in time to peak tension, varied with the calcium level in just the same way as the length-tension relation for developed tension (Fig. 3b). The argument that tension changes in parallel elastic structures might have interfered in some way with our measurements can also be dismissed because the difference we have noted in the form of the length-tension relation is present at lengths where there is no resting tension (Fig. 3a). We have also considered the possibility that the change seen in the normalised curve could be due to the presence of an internal load that increases in size as the muscle length decreases (such a load must exist because the relaxed length of a muscle is greater than the shortest length to which it can contract). Although

this is a possible explanation, quantitative estimates of the size of the load required to produce the observed shift in the normalised curves are not consistent when two of the curves in Fig. 3b are used to predict the shift in the third one; furthermore, the size of the load required to produce the observed effects decreases rather than increases at shorter muscle lengths.

Thus, it appears from Fig. 1 and the results shown in Fig. 3 that in cardiac muscle, as in frog skeletal muscle under some conditions (such as tetanic contractions at short muscle lengths², twitch contractions at room temperature⁷), the form of the length-tension relation is not determined simply by the properties of the sliding filament system. In both types of muscle length-dependent activation processes appear to have an important influence on the form of the curve, at least at lengths below $L_{\max}$. As cardiac muscle is probably confined to working below $L_{\max}$ under physiological conditions because of its large resting elasticity, this is a matter of considerable practical importance, but at this stage the nature of the length-dependence of activation processes can only be a matter of speculation. The fact that peak tension is reached sooner at short muscle lengths (Fig. 2c) suggests that deactivation associated with shortening is occurring⁸, and observation of the muscles operating at the foot of their length-tension curve confirms that they are visibly slack at rest and must therefore shorten appreciably before exerting tension. Incomplete activation due to failure of the inward spread of excitation to centrally-placed myofibrils⁹ seems less likely in cardiac muscle than in skeletal muscle because of the much smaller diameter of the cardiac muscle cell.

It seems that in heart muscle in contrast to skeletal muscle the rise in intracellular Ca^{2+} concentration produced by each action potential is insufficient to give full activation of the contractile system. The degree of activation is thought to be affected by the rate of stimulation and extracellular Ca^{2+} concentration¹⁰, the duration of the action potential¹¹, and the effect of various drugs¹²; and the observations reported here provide evidence that muscle length is another factor of importance in determining the completeness of the excitation-contraction coupling process.

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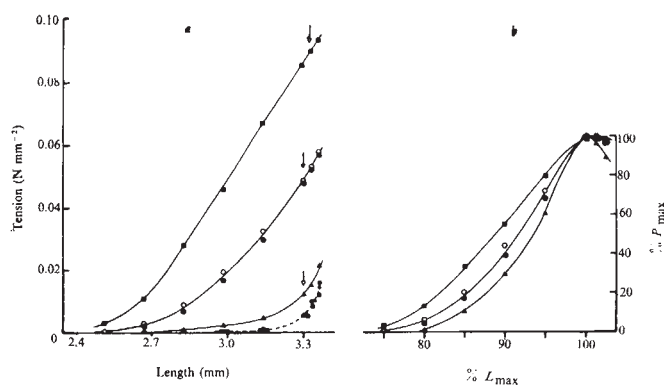


Fig. 3 a, Length-tension curves at three different calcium concentrations (same preparation as Fig. 2) ■, 8 mM Ca^{2+} ; ○, first series at 2.0 mM Ca^{2+} ; ●, repeat series after the determinations at 0.5 and 8 mM Ca^{2+} ; ▲, 0.5 mM Ca^{2+} ; —, peak twitch tension; ---, resting tension. The arrows indicate $L_{\max}$ for each curve. b, Normalised length-tension curves at three different calcium concentrations. The ordinate is developed tension as % $P_{\max}$ and the abscissa is the length as % $L_{\max}$. Symbols as in a.

- ¹ Gordon, A. M., Huxley, A. F., and Julian, F. J., *J. Physiol. Lond.*, **184**, 170 (1966).
- ² Taylor, S. R., and Rudel, R., *Science*, **167**, 882 (1970).
- ³ Fawcett, D. W., and McNutt, N. S., *J. Cell Biol.*, **42**, 1 (1969).
- ⁴ Fisher, V. J., Lee, R. J., Marlon, A. M., and Kavalier, F., *Circulation Res.*, **20**, 520 (1967).
- ⁵ Jewell, B. R., and Rovell, J. M., *J. Physiol. Lond.*, **235**, 715 (1973).
- ⁶ Blinks, J. R., and Jewell, B. R., in *Cardiovascular Fluid Dynamics*, **1**, 225 (1972).
- ⁷ Close, R. I., *J. Physiol., Lond.*, **220**, 745 (1972).
- ⁸ Jewell, B. R., and Wilkie, D. R., *J. Physiol., Lond.*, **152**, 30 (1960).
- ⁹ Gonzalez-Serratos, H., *J. Physiol., Lond.*, **212**, 777 (1971).
- ¹⁰ Winegrad, S., and Shanes, A. M., *J. gen. Physiol.*, **45**, 371 (1962).
- ¹¹ Kaufmann, R. L., Antoni, H., Hennekes, R., Jacob, R., Kohlhardt, M., and Lab, M. J., *Cardiovascular Res. Suppl.* **1**, 64 (1971).
- ¹² Naylor, W. G., *Circulation Res., Suppl.* **III**, 21, 213 (1967).

Pattern reconstitution in abdominal segment of *Leucophaea maderae* (Blattaria)

DURING the past few years the cuticular patterns of insect segments has been looked at with increasing interest. Following transplantations of pieces of epidermis¹⁻⁵ all these patterns become remodelled in a characteristic way. Analysis of the patterns led to the idea of a segmentally reiterated gradient. According to this concept any line of a segment with its specific structure is characterised and possibly determined by a specific level of the gradient.

Many of the experiments dealing with the problem of the mutual correlations between the segmental gradient and pattern formation have been performed in the bug *Rhodnius*^{2,6}. In this species the cuticular pattern of the tergites consists of medio-laterally oriented surface ripples. When square pieces of the integument are cut out and rotated through 90° the orientation of cuticular structures in the transplant gradually approaches the normal orientation during the following moults.

There are two main ways this reconstitution of the pattern could occur. (1) It is assumed that the gradient has many of the properties of a concentration-diffusion gradient. A cell differentiates and secretes its specific cuticle according to the level at which it happens to lie. A change of the level of the gradient in the surroundings of the cell is followed by a change of the differentiation properties of the cell; the cell is 'reset' to the new level. Thus the reconstitution of the pattern after a 90° rotation is due to the levelling of discontinuities of the gradient by processes similar to diffusion and the subsequent redifferentiation of the cells. The cells of the transplant remain in their rotated position but change their polarity and pattern. Computer simulations based on these propositions showed agreement with the observed cuticular patterns.⁶ (2) It is assumed that the gradient features and the differentiation properties of the cells are more stable and cannot be changed by diffusion-like processes. The approximation of the pattern towards normality after the 90° rotation could be brought about by a bodily rerotation of the whole transplant. In this case the cells of the transplant would change their position relative to the host tissues but preserve their original differentiation properties. Similar rerotations of grafts had been observed in the eyes of amphibia⁷ and in the legs of cockroaches⁸.

In *Rhodnius* there are no proper epidermal and cuticular markers which could allow a clear decision between the two alternatives. So it seemed desirable to make similar experiments in species better suited for this purpose. This and the following two papers describe rotation experiments performed on the tergites of two hemipteran species and of a cockroach⁵.

Larvae of *Leucophaea maderae* were used for the experiments. The cuticular pattern of the larval tergites consists of two areas: the anterior half of the tergite has a more or less smooth cuticle with only a few tiny bristles; the posterior half has a cuticle with many tubercles of different size in a regular but individually variable distribution. The two fields are separated by a prominent transversal ridge. Square pieces of integument containing the ridge and parts of both regions were cut out and reimplanted after a rotation through 90°. The angle between the ridge of the transplant and a reference line in the host segment was measured on the mounted cuticles of the successive larval instars up to the imago.

As shown in Fig 1a-f the angle between the ridge of the transplant and the reference line diminishes gradually during the following moults. The bristles and tubercles in the central area of the transplant stay in a more or less constant position relative to the ridge (Fig 1g-k). Thus the whole pattern rotates back towards the original position.

The pattern of bristles and tubercles on the tergites of

Leucophaea is highly variable; no two segments have an identical pattern. If it is assumed that after the rotation of the transplant the cells are reset to new gradient levels and according to their new levels generate a new pattern one would expect that the distribution of bristles and tubercles in the newly formed pattern would be different from that in the original. The observation of an identical pattern rotating back towards the original position is a strong indication for a rerotation of the transplant tissues as a whole. The entire pattern changes are not to be explained purely

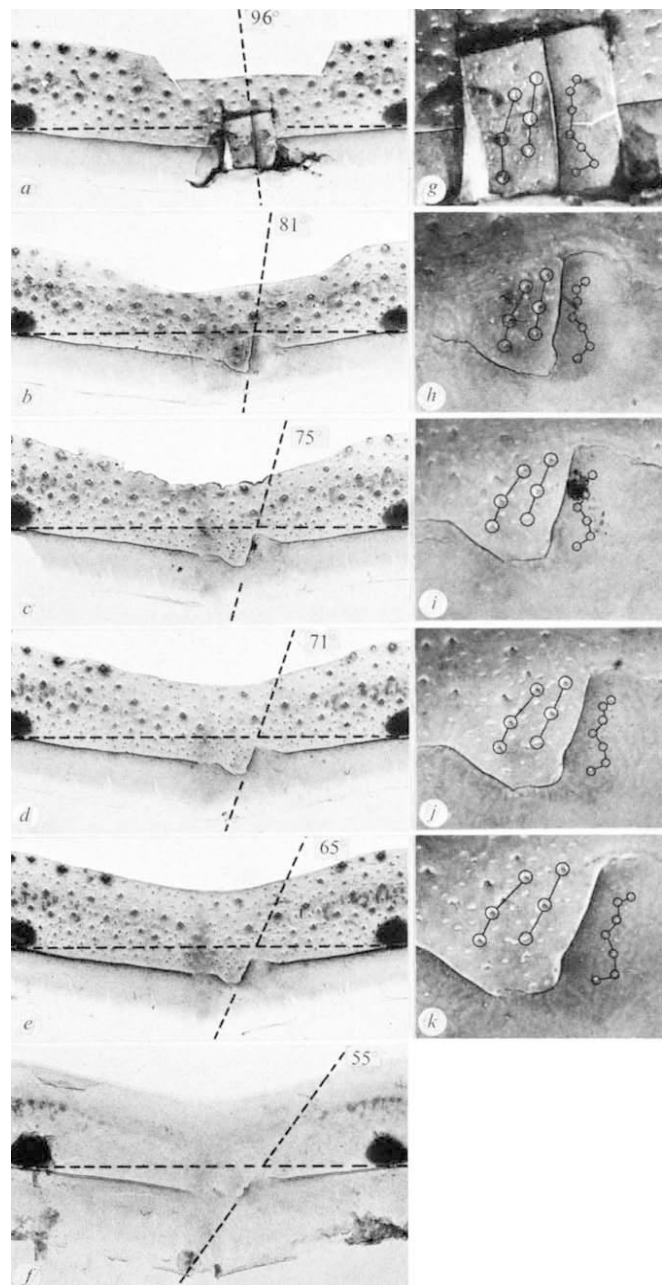


FIG. 1 a-f, Development of an anticlockwise rotated piece of skin during the following moults up to the imago. The cuticle of one instar (between d and e) had been lost. The angle between the ridge of the transplant and a reference line (between insertions of left and right dorso-ventral muscles) diminishes from 96° to 55°. At the right (g-k) the corresponding region of the transplant is shown at a higher magnification. Part of the bristles and tubercles are outlined by circles to demonstrate that the relative positions of ridge, bristles and tubercles of most parts of the transplant remain more or less unchanged; the pattern as a whole rotates back. Magnifications: a, $\times 101$; b, $\times 92$; c, $\times 83$; d, $\times 76$; e, $\times 51$; f, $\times 51$; g-k, $\times 303$.

by rotation however, since this could not explain how the ridge of the transplant joins up with that of the host. The generation of intermediate gradient values by intercalary regeneration is one possible explanation.

Though it is only the pattern on the cuticle whose rotation can be observed in *Leucophaea* there is no doubt that the epidermis of the transplant, too, rotates back since the cuticle is only a secretion product of the epidermis. More direct evidence for the movement of the epidermis is presented in the following contributions^{9,10}. So pattern reconstitution in the tergites of *Leucophaea* is, at least mainly, brought about by a rerotation of the transplant tissues; it is not necessary to assume a resetting of the cells. In this respect the epidermis of the tergites is like the legs in which a resetting to new levels seems to be possible only in proliferating tissues⁵.

The mechanics of the rerotation has not been studied in detail up to now. Muscle contraction as a means for movement can be excluded since the transplant region is completely free of muscles. Most likely the rotation is achieved by an active movement of the border cells, while the more central parts are carried along with them passively as can be judged from the unchanged pattern of large central parts (Fig. 1g-h; see also ref. 10). Pushing the transplant by intercalary regeneration should also be taken into account.

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- ¹ Marcus, W., *Wilhelm Roux Arch. Entw.-Mech. Org.*, **154**, 56 (1962).
- ² Locke, M., *J. exp. Biol.*, **36**, 459 (1959).
- ³ Stumpf, H. F., *J. exp. Biol.*, **49**, 49 (1968).
- ⁴ Lawrence, P. A., *J. exp. Biol.*, **44**, 607 (1966).
- ⁵ Bohn, H., *Wilhelm Roux Arch. Entw.-Mech. Org.*, **165**, 303 (1970).
- ⁶ Lawrence, P. A., Crick, F. H. C., and Munro, M., *J. Cell Sci.*, **11**, 815 (1972).
- ⁷ Hunt, R. K., and Jacobson, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 507 (1973).
- ⁸ Bohn, H., *Wilhelm Roux Arch. Entw.-Mech. Org.*, **156**, 449 (1965).
- ⁹ Lawrence, P. A., *Nature*, **248**, 609 (1974).
- ¹⁰ Nübler-Jung, K., *Nature*, **248**, 610 (1974).

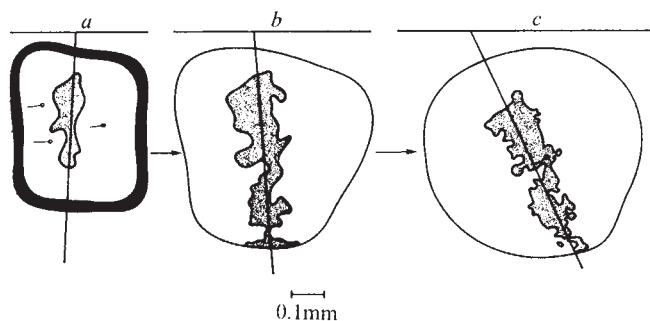


FIG. 1 Camera lucida drawings showing the result of transplanting a piece of orange epidermis containing a transparent clone (shaded) on to a white fourth-stage host. The graft was rotated through 90° clockwise. a, The situation immediately on grafting (clone rotated c. 93°); b, the young fifth-stage (angle of clone now c. 85°); c, the adult (angle of clone now about 65°).

cuticular patterns were almost identical. We therefore suggested that the cells' set level was re-established at some stage in the cell division cycle allowing further diffusion and consequent changed polarity. An alternative cause of pattern change could be bodily rotation of the entire graft in the opposite direction to that of the original experiment⁹. This paper and the two accompanying contributions^{3,4} show that in fact there is bodily rotation of the graft.

Pieces of epidermis were exchanged between wild type (orange pigmented cells) and a mutant, *wb* (white pigmented cells) of *Oncopeltus fasciatus*⁵. The adult hairs and cuticular tubercles were used as polarity indicators, both normally

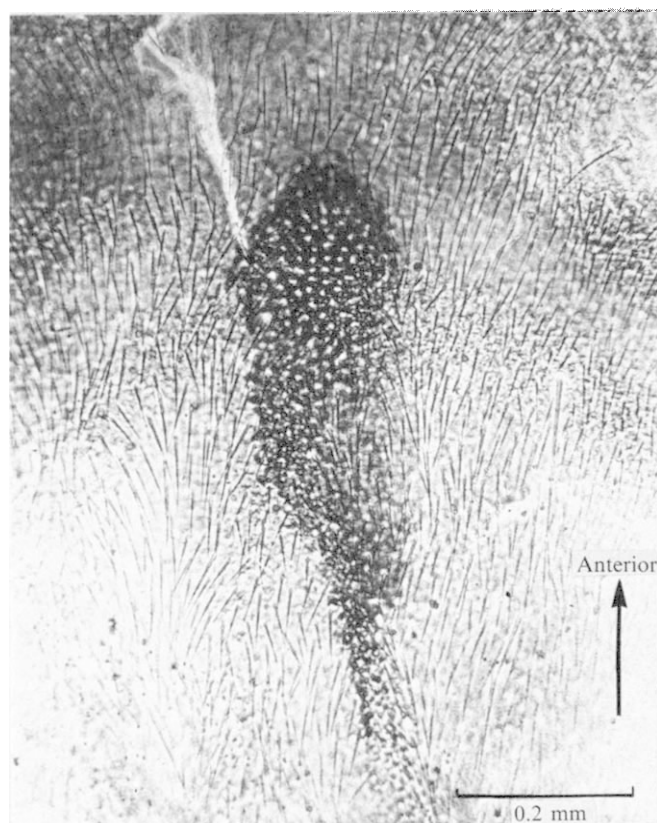


FIG. 2 A strip of orange epidermis (long axis mediolateral) was rotated through 90° clockwise and transplanted on to a white fourth-stage larva. In the adult the strip of orange cells is still clearly visible and still retains the same orientation as it had on grafting (long axis antero-posterior). As in the host, the hairs in the graft point posteriorly.

Cell movement during pattern regulation in *Oncopeltus*

THE cuticle of adult *Rhodnius* (Hemiptera) is marked by mediolaterally oriented surface ripples. From transplantation and rotation experiments on the epidermis Locke¹ demonstrated a segmentally reiterated gradient. We² have proposed a model of the gradient which is considered as a concentration of some diffusible substance, the direction of steepest slope of concentration specifying cellular polarity and thereby the orientation of the ripples. We suggested that the cells might contribute to the stability of the gradient and attempt to maintain their original or 'set' level when transposed.

Rotation of a larval square of epidermis through 90° results in an S-shaped pattern of ripples in the adult cuticle. When such an adult is made to moult again to produce a second cuticle the S-shaped pattern flattens, so that the ripples are now aligned nearer to the mediolateral axis. This change of pattern was associated with the presence of cell divisions in the artificial moult cycle; when the moult cycle was truncated and cell divisions omitted, the first and second

pointing posteriorly. Following X-irradiation as eggs⁶, fourth-stage larvae were screened for long thin clones which were a different colour than orange and had their long axes parallel to the mediolateral axis of the body. Square pieces of epidermis containing such clones were excised, rotated through 90° and implanted on a white host in the centre of the segment. The graft border and shape and orientation of the clone were drawn by camera lucida in the two succeeding instars (Fig. 1). In some cases the clone was damaged by the operation but in all five cases where the clone survived as a coloured strip, there was clear evidence for the rotation of the clone and therefore for bodily rotation of the graft; in no case did the central hairs become reorientated while the clone remained in the original orientation. In one case the clone itself became S shaped suggesting more cell movement at the edges of the graft.

My unpublished experiments on *Dysdercus fasciatus*, and those of Nübler-Jung⁴ on *D. intermedius* suggest that the rotation of grafts is restricted to a relatively short period (about 20 h⁴) when the epidermis separates from the cuticle; that is, after cell divisions have ceased.

In a second series of experiments (suggested to me by Dr H. Bohn) thin strips of epidermis, with their long axes oriented mediolaterally were taken from orange *Oncopeltus* larvae, rotated through 90° and transplanted on to white larvae. When the graft survived as a strip, it remained oriented close to the anteroposterior axis, but the orientation of the hairs, and the small epidermal tubercles turned so that they pointed posteriorly (Fig. 2).

These two classes of experiments show that the situation is complex; the first shows that cell movement is an important component of pattern regulation, and the second that regulative changes of polarity can occur without bodily rotation of the entire graft. It is still possible that regulation of polarity is achieved by smaller parts of the graft rotating independently. Rotation back of grafts has been demonstrated in other systems (the amphibian ear⁷ and eye⁸, and the insect leg⁹), while polarity changes that are not dependent on rotation are found after juxtapositions of regions from different levels in the axis of both the abdominal segments² and the legs of insects⁹. These changes are associated with cell division.

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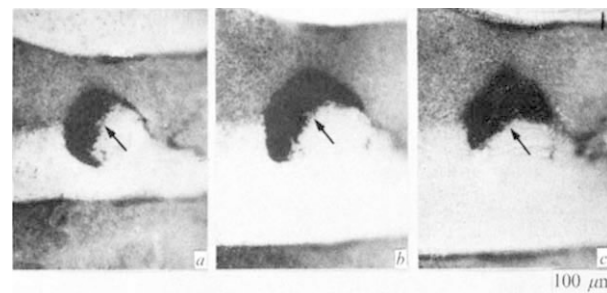


FIG. 1 Development of a transplant rotated anticlockwise through 90° during the third larval instar. *a*, Aspect early in the fourth instar; *b*, late fourth to early fifth instar; *c*, shortly before metamorphosis. Posterior segment border at the bottom. Scale, 100 μm.

posed cells.³ Until now it was not possible to decide between these two mechanisms since pattern reconstitution in the leg or body segment was studied only by the observation of cuticular structures such as bristles and ripples⁴. These structures reflect the physiological status of the epidermal cells only during the short periods of epicuticle formation but not during the intervals in between. Moreover, by studying the cuticle it is rarely possible to determine the exact boundary between host and transplanted epidermis. These disadvantages can be overcome by using a colour mutant⁵ which enables continuous observation of the transplanted cells.

In the wild type of *Dysdercus intermedius* from the third larval instar onwards the anterior zone of the third ventral abdominal segment is red and the posterior zone white. The mutant lacks the red pigment and therefore the anterior zone looks grey. It should be noted that the pigment is located inside the epidermal cells. The cuticle is transparent and permits direct observation of the location and possible movements of the transplanted cells. Transplantations were carried out on third instar larvae as described by Lawrence *et al.*³. The transplants always included the border between the red and white zones. The hosts were of the grey mutant type. The segments carrying transplants were filmed by time-lapse cinematography (1 frame per 3 min) or photographed at daily intervals until after metamorphosis.

Three stages in the development of a transplant which had been rotated anticlockwise through 90° are illustrated in Fig. 1. After the first moult following the operation (Fig. 1*a*) some red cells are found between the white cells of the graft and

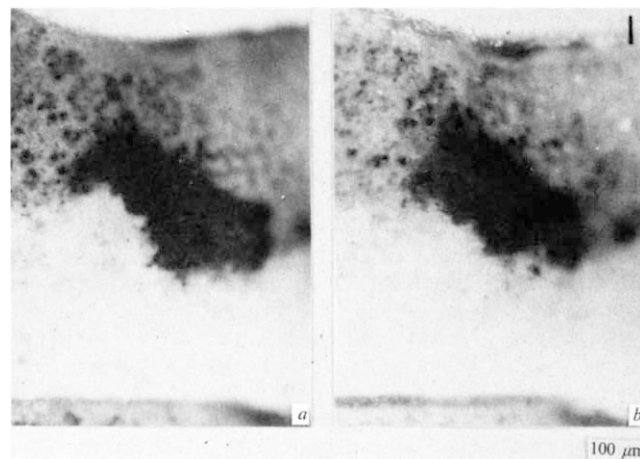


FIG. 2 Time-lapse film which was taken towards the end of the fifth larval instar. *a*, Aspect at the beginning of film (0 h); *b*, at the end (54 h) of the film. Scale, 100 μm.

¹ Locke, M., *J. exp. Biol.*, **36**, 459 (1959).

² Lawrence, P. A., Crick, F. H. C., and Munro, M., *J. cell Sci.*, **11**, 815 (1972).

³ Bohn, H., *Nature*, **248**, 608 (1974).

⁴ Nübler-Jung, K., *Nature*, **248**, 610 (1974).

⁵ Lawrence, P. A., *Genet. Res. Camb.*, **15**, 347 (1970).

⁶ Lawrence, P. A., *J. embryol. exp. Morph.*, **30**, 681 (1973).

⁷ Ogawa, C., *J. exp. Zool.*, **34**, 17 (1921).

⁸ Hunt, R. K., and Jacobson, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 507 (1973).

⁹ Bohn, H., *Wilhelm Roux Arch. Entw.-Mech. Org.*, **156**, 449 (1965).

Cell migration during pattern reconstitution in the insect segment (*Dysdercus intermedius* Dist., Heteroptera)

If in the insect segment a piece of epidermis is rotated through 90° the changed pattern tends to return to normal after several moults.¹ This observation can be explained by a rotation of the whole transplant (ref. 2 and H. Bohn, personal communication) or by a resetting of the trans-

the grey cells of the host (upper right). This aspect remains stationary for several days while the larva grows and mitoses occur in the epidermis⁸. When the epidermis is detached from the old cuticle in preparation for the next moult the spur at the posterior end of the red patch disappears and the wedge of red cells at the right anterior rim increases considerably in width. This aspect (Fig. 1b) persists through moulting and during the following days. Some days before metamorphosis, when the epidermis again detaches from the cuticle, the posterior region seems to move forward and the wedge at the right is strengthened again. Simultaneously, the angle between the posterior segment margin (at the bottom) and the red/white border within the transplant decreases further. The resulting pattern (Fig. 1c) is retained by the imago. It should be noted that some local peculiarities at the red/white border within the transplant (arrows) persist throughout development. During all these changes no pink cells were observed which might indicate a change of pigment synthesising activities.

The analysis of time-lapse films (Figs 2 and 3) reveals that the rotation of the red/white border with respect to the posterior segment margin and the concomitant changes in shape are accomplished within 20 to 25 h ($28 \pm 1^\circ\text{C}$).

These observations demonstrate clearly that the transplanted cells may change their positions beneath the cuticle with respect to landmarks on the host segment, while at the same time retaining to some extent their relative positions within the transplant (arrows in Fig. 1). The running time-lapse films give the impression that the transplanted cells near the margin move considerably during the motile phase. In bigger transplants the more central regions seem to be

rotated passively. This impression is compatible with the data derived by scoring several parameters at intervals (Fig. 3a and b). It therefore seems possible to explain pattern reconstitution in *Dysdercus* without assuming some kind of resetting (in this case of pigment synthesis) in the transplanted cells. Finally it may be pointed out that the change of the angle between segment margin and the red/white border corresponds to changes in ripple orientation described by Lawrence *et al.*³ in *Rhodnius*. Should the changes in this insect also be due to cell migration in the marginal parts and rotation of the central parts of the transplant, then some inconsistencies between the observed patterns and those simulated by the computer on the basis of the resetting hypothesis (their Figs 8a and 19) might be due to migration of the marginal transplant and perhaps host cells.

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¹ Locke, M., *Adv. Morphogen.*, **6**, 33 (1967).

² Bohn, H., *Wilhelm Roux Arch. Entw.-Mech. Org.*, **156**, 449 (1965).

³ Lawrence, P. A., Crick, F. H. C., and Munro, M., *J. cell Sci.*, **11**, 815 (1972).

⁴ Lawrence, P. A., *Adv. Insect Physiol.*, **7**, 197 (1970).

⁵ Hollweg, G., *Biol. zbl.*, **91**, 545 (1972).

⁶ Lawrence, P. A., *J. Cell Sci.*, **3**, 391 (1968).

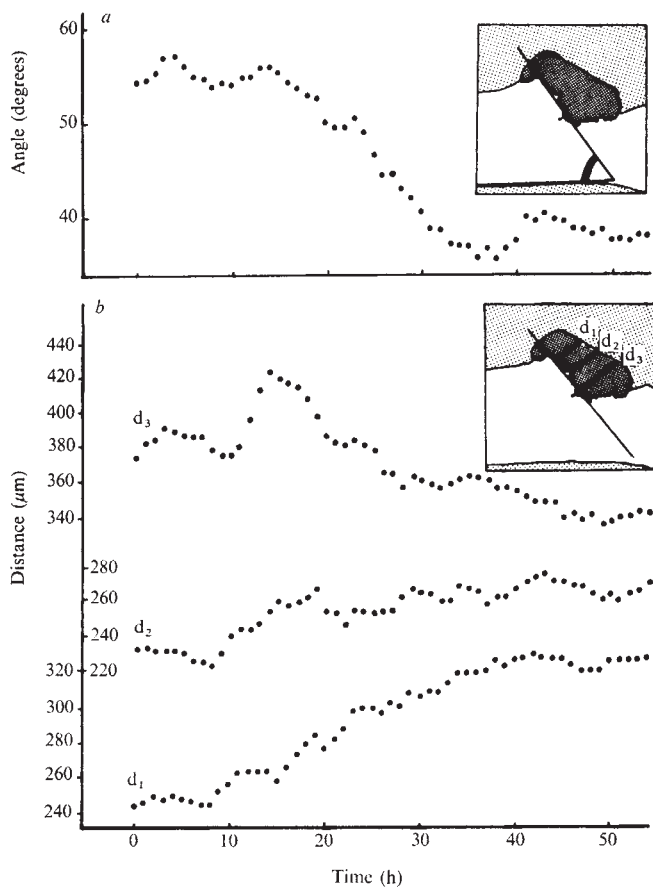


Fig. 3 Analysis of the time lapse film mentioned in Fig. 2. a, Change of the angle between the red/white border and the posterior segment margin; b, change of the dimensions of the red patch along the lines marked as d_1 , d_2 and d_3 (see inset, cf. Fig. 2a). The curves are smoothed by running averaging over three terms.

Left-handed subunit helix in flagellar microtubules

ONE of the problems encountered in the study of a small negatively stained biological object by electron microscopy concerns the uncertainty in the distribution of stain around the object. If the particle is considered broadly as having two 'sides' one in contact with the support film and the other not, then the staining conditions may produce an image which represents one or other of the two surfaces or both sides together. Generally, the degree of contrast lies somewhere between these extreme cases¹. Unless there is a differential staining of one side with respect to the other and an unambiguous distinction between the near and far sides can be made it is generally impossible to determine the right or left-handedness of an object. (Moody² has however been able to demonstrate the right-handed sense of bacteriophage T4 sheath by the use of a convenient staining artefact).

A straightforward method for determining the sense of a helical object has been described recently by Finch³ in an investigation of negatively stained TMV particles, and in the present study this technique has been applied to the microtubules from the flagella of *Chlamydomonas reinhardtii*. The method depends on the fact that as a helix is tilted about an axis perpendicular to its own axis, either towards or away from an observer, the projected image becomes asymmetric about the centre line. More precisely, the helix lines at the edge of the image on one side become more inclined with respect to the axis and less so on the other. The difference in appearance between the two sides is most marked when the tilt is equal to the pitch angle of the helix which is, for a

microtubule, approximately 12° – 15° . This is the most obvious helix in the negatively stained structure and is defined as that with the smallest pitch passing through subunits in adjacent longitudinal filaments⁴.

A short section of central microtubule from a *Chlamydomonas* flagellum is shown in Fig 1. The microtubule, negatively stained with uranyl acetate, has been tilted towards (positive) the observer by 12° in the upper micrograph and in the opposite direction (negative) in the lower micrograph. The top end of the microtubule, as it appears in the

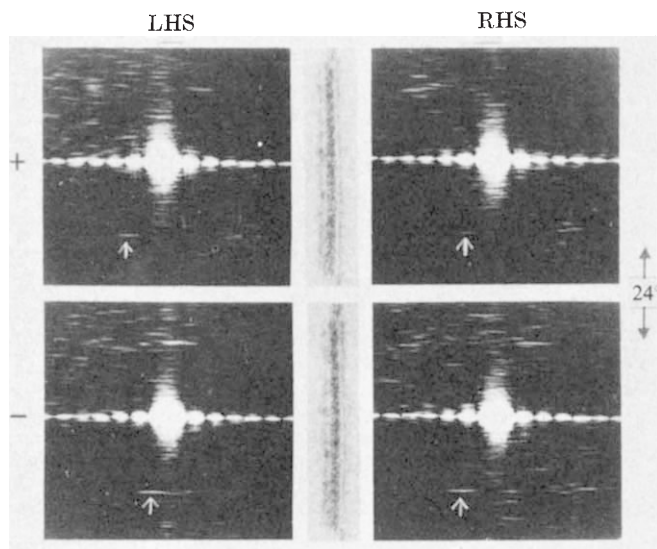


FIG. 1 A short section of negatively stained central microtubule from a *Chlamydomonas* flagellum tilted, by 12° , towards (+) and away (–) from the observer. The optical transforms are of the right (RHS) and left (LHS) hand side of each micrograph and the major 4 nm reflection is shown in each by an arrow. The reflection lies closest to the meridian for RHS+ and LHS– (particularly clear for the negative tilt) indicating a left-handed helix of subunits.

figure, moves towards the observer for positive tilt and away for negative tilt. Clearly the micrographs themselves do not reveal any distinctive change in pattern across the microtubule diameter but optical transforms of each half (RHS and LHS) of the two images are capable of resolving the differences. A strong reflection from the 4 nm axial periodicity in the microtubule^{4,5} is arrowed in each transform and the radial positions may be compared in the two transforms from each micrograph. (The presence of only one strong reflection close to the meridian in each pattern signifies predominant staining of only one side of the microtubule.) It can be seen that the reflection lies closest to the meridian for RHS positive and LHS negative and this is compatible with a left-handed helix³. The differences are small because of the flattening of the microtubule but are, nevertheless, distinct. Additional reflections further away from the meridian appear in some of the transforms and their relative positions also change with the degree of tilt. These can also, in principle, be used to determine the helix sense but as they correspond to families of subunit helices of greater pitch, the microtubule would have to be tilted through a considerable angle (40° – 50°) before any measurable change could be detected in the radial positions of the diffraction maxima. Since the image quality deteriorates rapidly with increasing tilt angle observations have to be restricted to the near-meridional reflections. Of the transforms so far examined those that contain discrete 4 nm reflections have shown consistently a left-handed helix of subunits, although no conclusive results have as yet been obtained for microtubules from sea urchin sperm flagella.

Once the helix sense has been determined it is also possible to infer from the 'sense' of the transforms which side of the microtubule is stained in a one-sided image. The diffraction patterns in Fig 1, and the majority of those obtained, indicate for this preparation predominant staining of the surface away from the specimen grid.

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¹ Klug, A., and Berger, J. E., *J. molec. Biol.*, **10**, 565 (1964).

² Moody, M. F., *J. molec. Biol.*, **25**, 167 (1967).

³ Finch, J. T., *J. molec. Biol.*, **66**, 291 (1972).

⁴ Chasey, D., *Expl. Cell Res.*, **74**, 140 (1972).

⁵ Grimstone, A. V., and Klug, A., *J. Cell. Sci.*, **1**, 351 (1966).

Suppressor cells in normal immunisation as a basic homeostatic phenomenon

WHEN guinea pigs are treated with one dose of cyclophosphamide (CY, 300 mg kg⁻¹) 3 d before sensitisation with either ovalbumin in Freund's incomplete adjuvant (OA/FIA) or 2,4 dinitrofluorobenzene (DNFB), the resultant Jones-Mote or contact skin reactions elicited 1 week later are increased in intensity and induration and prolonged as compared to control animals^{1,2}. We have postulated that CY pretreatment depletes a population of cells which normally modulate contact sensitivity and Jones-Mote reactions^{1,2}. If CY was depleting a population of suppressor cells normally present at the time of sensitisation, the passively transferred cells from these animals should be more reactive than if there was no CY given to the donors. Passive transfer of spleen or peritoneal exudate cells (PEC) from animals sensitised with OA/FIA 8 d earlier caused more intense and more indurated reactions in normal recipients when donors had been pretreated with CY 3 d before the OA/FIA sensitisation, when compared with those reactions transferred from sensitised animals which had not received CY (S. I. K., D. P., and J. L. T., unpublished) (Fig. 1). We used the following model for the assay of suppressor cells. Guinea pigs, treated with CY 3 d before immunisation with OA/FIA, served as cell recipients 8 d later. They were then potentially susceptible to the effect of suppressor cells which they were lacking.

The recipient animals in all experiments were 450–500 g albino Hartley strain guinea pigs. They were all treated

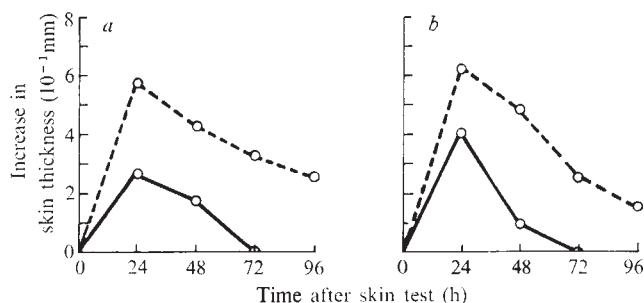


FIG. 1 Passive transfer into normal animals of cells 8 d after sensitisation with OA in FIA with (○—○) or without (○---○) CY pretreatment. There were five recipients in each group. a, spleen cells; b, PEC.

TABLE 1 Skin reactivity of animals treated with CY 3 d before sensitisation with OA in FIA, serving as cell recipients 8 d after immunisation

Donor immunised with	Days after sensitisation donor cells transferred	CY pretreatment of donor	Viable No. cells Spleen	PEC	No. of recipients	Increase in skin thickness*
No donor	—	—	—	—	11	4.6
OA/FIA	8	No	3×10^8	—	8	2.6
OA/FIA	8	No	—	2×10^8	8	1.8
OA/FIA	8	Yes	3×10^8	2×10^8	5	6.7
OA/FIA	8	Yes	—	2×10^8	5	6.0
BGG/FIA	8	No	3×10^8	—	5	5.2
BGG/FIA	8	No	—	2×10^8	5	5.2
OA/FIA	14	No	3×10^8	—	5	1.7
OA/FIA	14	No	—	2×10^8	6	1.9
OA/FCA	8	No	3×10^8	—	5	1.8
OA/FCA	8	No	—	2×10^8	5	1.5
OA/FIA	8	No	0†	—	5	5.4
OA/FIA	8	No	—	0†	4	5.0

* 24 h after skin test with 100 μ g OA (10^{-1} mm).† 3×10^8 spleen cells and 2×10^8 PEC heat killed before transfer.

with 300 mg kg^{-1} CY intraperitoneally (LD_{50-20}) 3 d before footpad injection with 1 μ g OA in FIA (evenly divided in each footpad). Eight days after sensitisation, these animals received an intravenous injection of thrice washed spleen cells (3×10^8 viable) or PEC (2×10^8 viable) from sensitised donors. Skin tests with 100 μ g OA were performed 1 h after cell transfer and the skin test reactivity was assessed as described previously³. One group of CY treated OA/FIA sensitised animals always served as controls and did not receive any cells.

When recipient animals received either spleen cells or PEC from donors sensitised with 1 μ g OA in FIA 8 d before, the skin test reactions were markedly reduced as compared to control animals not receiving cells and to those recipients receiving cells from animals sensitised with 1 μ g bovine gamma globulin (BGG) in FIA 8 d before (Fig. 2). When the donor animals were treated with 300 mg kg^{-1} CY 3 d before immunisation with OA/FIA and the cells taken for transfer 8 d later, the skin tests of the recipient animals were increased as compared with the control animals not receiving cells and with the animals receiving cells from OA/FIA sensitised animals which had not received CY (Fig. 3). This is explicable on the assumption that, as a result of CY pretreatment of the donors, the transferred cells lacked a population of suppressor cells. The increase in reactivity would therefore result from the transfusion of effector cells alone. The results of all assays for suppressor cells using this system are shown in Table 1. Cells taken from animals 14 d after immunisation with OA in FIA and transferred into CY pretreated OA/FIA animals also decreased the reactivity of the recipient animals. Cells taken from animals immunised with 10 μ g OA in Freund's complete adjuvant (FCA) suppressed the reactivity of the recipient animals. When cells from donors immunised 8 d earlier with OA/FIA were killed by heating at 56° C for 40 min (spleen < 1% viable, PEC < 2% viable as assessed by Trypan blue exclusion) they did not suppress the reactivity of the recipient animals. Other control experiments demonstrated that cells taken from OA/FIA animals had the same reactivity in recipients whether or not the recipients were treated with CY 11 d before cell transfer.

These findings demonstrate that during normal sensitisation with OA in FIA or FCA, suppressor as well as effector cells are stimulated. The suppression of skin test reactivity in this system depends on live cells and is immunologically specific. Cells from animals sensitised to OA in FIA 7 or 8 d previously can transfer Jones-Mote type reactions to normal animals. The reactions observed start 6–8 h after skin test, reach a peak at 24 h and are markedly reduced or gone at 48 h.

It is difficult to demonstrate delayed hypersensitivity (Jones-Mote) reactions in animals sensitised with OA/FIA 14 d previously as, owing to the presence of humoral antibody, they show strong Arthus reactivity. Lymphocytes from these animals cannot transfer delayed type reactivity. If suppressor cells and antibody production are not allowed to develop as a result of CY pretreatment, however, guinea pigs show delayed hypersensitivity which can be transferred with lymphocytes to normal recipients (S. I. K., D. P., and J. L. T., unpublished). Thus, using the optimal immunisation schedule for the generation of Jones-Mote reactions⁴, effector as well as suppressor cells are normally demonstrable 7 or 8 d after sensitisation, whereas the balance of suppressor to effector cells 14 d after immunisation may be such that no delayed hypersensitivity reaction is demonstrable. This effector-suppressor balance may explain the evanescence of the Jones-Mote type of delayed hypersensitivity reactions. The demonstration of cells able to suppress delayed hypersensitivity reactions 14 d after sensitisation with OA in FIA may also explain the findings of Loewi, Holborow and Temple⁵ who showed that split tolerance or immune deviation to OA (no delayed hyper-

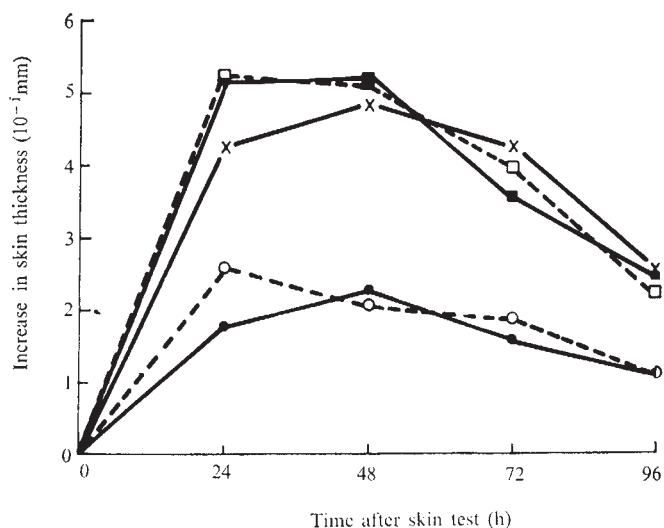


FIG. 2 Transfer of PEC and spleen cells. Donor cells taken 8 d after sensitisation with OA or BGG in FIA. Recipient animals, which were treated with CY 3 before immunisation with OA in FIA, received cells 8 d after immunisation. ●—●, OA/FIA PEC (8); ○---○, OA/FIA spleens (8); ×—×, controls (8); ■—■, BGG/FIA PEC (5); □---□, BGG/FIA spleens (5); () number of recipients.

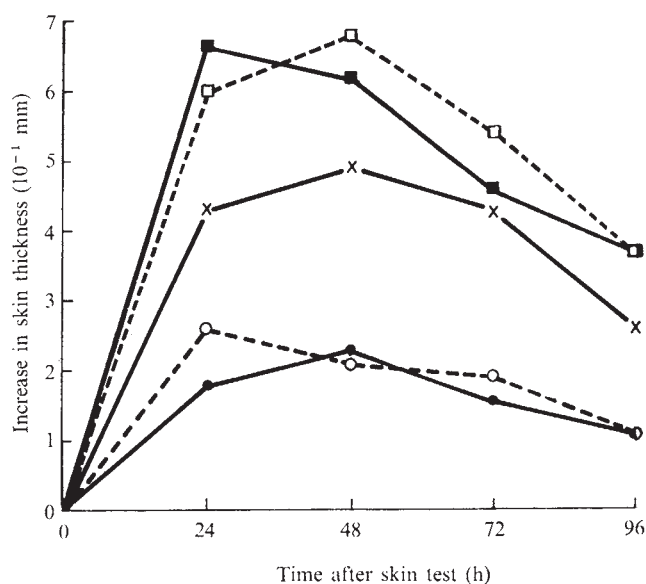


FIG. 3 Transfer of PEC and spleen cells. Donor cells taken 8 d after sensitisation with OA in FIA with or without CY pretreatment. Recipient animals, which were treated with CY 3 d before immunisation with OA in FIA, received cells 8 d after immunisation. •—•, OA/FIA PEC (8); ○—○, OA/FIA spleens (8); ×—×, controls (8); ■—■, CY—OA/FIA PEC (5); □—□, Cy—OA/FIA spleens (5). () number of recipients.

sensitivity reaction while antibody production is normal) follows 14 d after immunisation with OA in FIA. Although we had not anticipated the ability of cells from animals immunised with OA in FCA to suppress delayed hypersensitivity reactions, their presence is in keeping with our earlier studies². It may be that many more effector cells are stimulated by OA in FCA as compared to OA in FIA and when suppressor cells are depleted by CY pretreatment of OA/FCA animals the effect of this depletion is much more difficult to demonstrate.

What are these suppressor cells? Our earlier studies on the morphological and functional effects of one dose of CY show depletion of B cell areas from lymphoid tissues of mice and guinea pigs and a decrease in antibody formation^{1,2,6}. There is also a selective increase in θ -positive cells in CY-treated animals⁷. All this would indicate that CY has a selective effect on B lymphocytes and would favour the postulate that the suppressor cells are B cells as they are depleted with one dose of CY before sensitisation. CY may also affect short lived T lymphocytes however, and for this reason we thymectomised adult guinea pigs, sensitised them 6 weeks to 3 months later and found no change in skin test reactivity to OA or DNFB 7 d after sensitisation with OA/FIA or DNFB. By extrapolation from findings in the mouse, adult thymectomy should deplete short lived or T_1 cells⁸ and, if these were the suppressor cells, we should see changes in the skin test reactivity. The assumption that this procedure (adult thymectomy) should have the same effect in the guinea pig as in the mouse may be wrong.

The evidence thus favours the existence of B lymphocyte suppressor cells being stimulated during normal immunisation. These suppressor cells are acting at the efferent end of the delayed hypersensitivity reaction as skin tests are performed just 1 h after intravenous injection of cells. Several possibilities exist as to how this suppression may occur. First, in all the normal immunisation systems in which suppressor cells have been demonstrated there is eventual antibody production and it is possible that small amounts of antibody coat cells which then come into contact with the antigen and compete with effector cells for antigen. Moreover, it has not been possible so far to dissociate suppressor cell

from humoral antibody formation. Second, the suppressor cells may liberate substances which inhibit the effector cells or their products. Third, suppressor cells may liberate substances which are chemotactic for other cell types which might modulate the normal reaction. In this regard we have recently confirmed the work of Dvorak *et al.*⁹ who have demonstrated that basophils are found in high percentages in all of these skin test reactions when elicited early after immunisation.

Effective cell separation techniques for guinea pig cells will help determine, with greater certainty, which lymphocyte subpopulation is responsible for the suppression of delayed hypersensitivity reactions during normal immunisation. The mechanisms⁴ by which this suppression occurs could then be more easily determined.

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- 1 Turk, J. L., Parker, D., and Poulter, L. W., *Immunology*, **23**, 493 (1972).
- 2 Turk, J. L., and Parker, D., *Immunology*, **24**, 751 (1973).
- 3 Blazkovec, A. A., Sorkin, E., and Turk, J. L., *Int. Arch. Allergy*, **27**, 289 (1965).
- 4 Richerson, H. B., *J. exp. Med.*, **134**, 630 (1971).
- 5 Loewi, G., Holborow, E. J., and Temple, A., *Immunology*, **10**, 339 (1966).
- 6 Turk, J. L., and Poulter, L. W., *Clin. exp. Immun.*, **10**, 285 (1972).
- 7 Poulter, D. W., and Turk, J. L., *Nature, new Biol.*, **238**, 17 (1972).
- 8 Cantor, H., *Prog. Biophys. molec. Biol.*, **25**, 71 (1972).
- 9 Dvorak, H. F., Dvorak, A. M., Simpson, B. A., Richerson, H. B., Leskowitz, S., and Karnovsky, M. J., *J. exp. Med.*, **132**, 558 (1970).

Pyrrolizidine ester alkaloid in danaid butterflies

THE dihydropyrrolizines (I–III, Fig. 1a) found in the hair-pencil secretions of male butterflies of the sub-family Danaeinae^{1–5} and shown in one instance to be an important courtship pheromone⁶, are closely related to the hepatotoxic dihydropyrrolizine metabolites of the pyrrolizidine alkaloids^{7,8}. We have previously adduced evidence which points strongly to these hair-pencil constituents having their origin in plant alkaloids (or their metabolites) ingested by the adult insects⁵. Thus, there are a number of reports of adult male danaid butterflies being strongly attracted to, and sometimes feeding on, dead and withering plants containing pyrrolizidine alkaloids⁵. With one species, *Danaus chrysippus petilea* (Stoll) we have shown⁵ that when dihydropyrrolizine-deficient laboratory-reared butterflies are given access to one such alkaloid containing plant, *Heliotropium amplexicaule* (Vahl), they soon acquire the dihydropyrrolizine ketone (I) which is normally found on the hairpencils of this species.

We report here the first identification of a pyrrolizidine ester alkaloid, lycopsamine, in hair-pencil extracts of two Australian danaid species captured in the field. This alkaloid is typical of those occurring in the plants to which male danais show specific attraction. The additional molecular

complexity of the alkaloid compared with the dihydropyrrolizine pheromones and the fact that the type of branched chain acid esterifying the pyrrolizidine aminoalcohol is unique in nature to these plant alkaloids, leaves no doubt that the alkaloid is of plant origin. Its occurrence in hairpencil extracts is regarded as confirming that the dihydropyrrolizine pheromones are metabolites of plant alkaloids of the pyrrolizidine group.

Five male *Danaus hamatus hamatus* (Macleay) and twelve male *Euploea tulliolus tulliolus* (Fabricius) were captured at Wallaville, Queensland. The hairpencils of individual butterflies were removed and extracted with 30 μ l of methanol. The extracts were then individually examined, before and after conversion to trimethylsilyl derivatives, using an integrated gas chromatograph-mass spectrometer. The extracts of ten *Euploea tulliolus tulliolus* and all five *Danaus hamatus hamatus* contained the dihydropyrrolizines previously identified in these two species³ as well as a number of other components (Fig. 2). One component, common to the extracts of both species (Fig. 2a, peak 2 and Fig. 2b, peak 3) gave a mass spectrum corresponding to a viridifloric or trachelanthic acid ester of retronecine or heliotridine esterified at the C₆ hydroxyl.

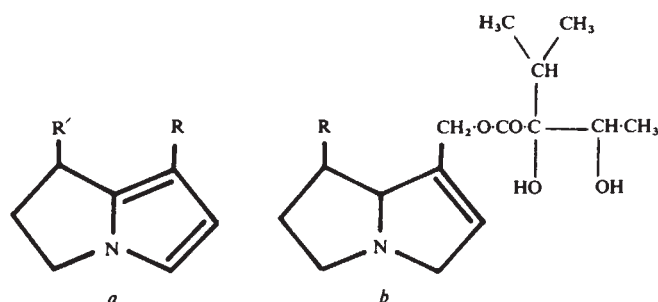


FIG. 1 a, The dihydropyrrolizines. I, R = $-\text{CH}_3$, R' = $=\text{O}$; II, R = $-\text{CHO}$, R' = $-\text{OH}$; III, R = $-\text{CHO}$, R' = $-\text{H}$. b, IV, R = β -OH ((-)-trachelanthic acid); V, R = β -OH ((+)-trachelanthic acid); VI, R = α -OH ((+)-trachelanthic acid); VII, R = β -OH ((+)-viridifloric acid); VIII, R = α -OH ((+)-viridifloric acid). (For explanation see text.)

Cochromatography of hairpencil extracts with authentic samples of the five known pyrrolizidine alkaloids (Fig. 1b) in this category⁹, that is indicine (retronecine, (-)-trachelanthic acid) (IV); intermedine (retronecine, (+)-trachelanthic acid) (V); rinderine (heliotridine, (+)-trachelanthic acid) (VI); lycopsamine (retronecine, (+)-viridifloric acid) (VII); and echinatine (heliotridine, (+)-viridifloric acid) (VIII), indicated that the butterfly alkaloid was lycopsamine (VII). The chromatographic retention time and the mass spectrum of the trimethylsilylated butterfly alkaloid also corresponded with those of authentic bis-trimethylsilyl lycopsamine.

Lycopsamine has been isolated from *Amsinckia hispida* (Ruiz et Pav.) I. M. Johnston, *A. intermedia* Fisch. et C. Mey., *A. lycopsoides* Lehm¹⁰ and *Echium lycopsis* L. (J. L. Frahn and J. A. Mills, personal communication). These plants are widely established in south-eastern Australia but occur either rarely (*Amsinckia*) or sparsely (*Echium*) in Queensland, mostly in the Darling Downs region. The known occurrences nearest to Wallaville are 180 miles distant for *Amsinckia* and 50 miles distant for *E. lycopsis* (S. L. Everist and V. K. Moriarty, personal communication). Other species which probably contain or are known to contain, this type of alkaloid occur more commonly in the Wallaville district. These include *Heliotropium amplexicaule* which is already known to attract adult male danaiids⁵ and to contain

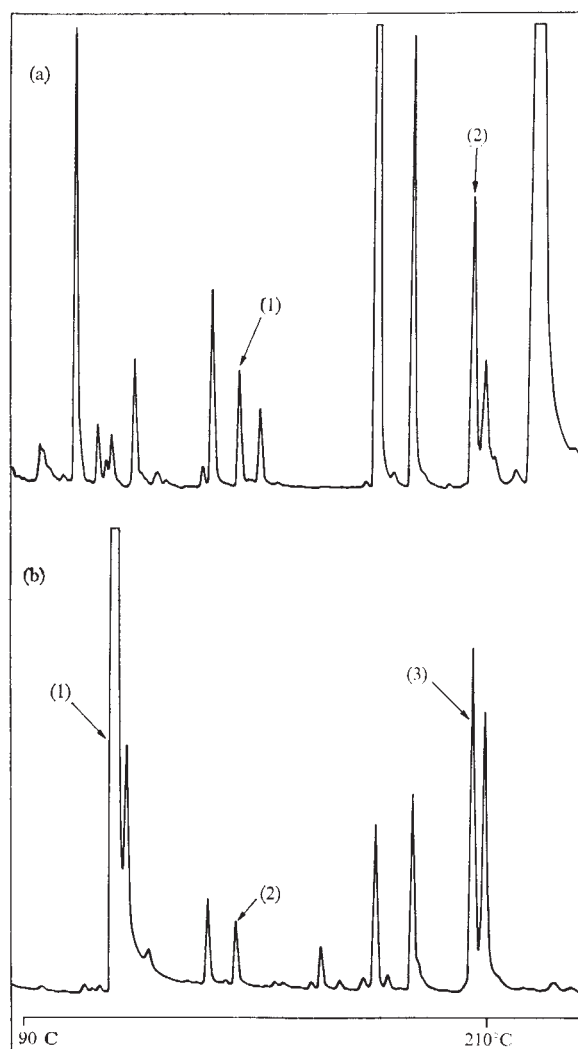


FIG. 2 a, Gas chromatogram typical of those obtained from hairpencil extracts of *E. tulliolus tulliolus* after trimethylsilylation using a 5 foot $\times$ 1/8 inch glass column packed with 2% SE30 on Chromosorb G, mesh size 80-100. Temperature programmed from 70°C to 210°C at 4°C min⁻¹. (1), The monotrimethylsilyl derivative of 1-formyl-7-hydroxy-6, 7-dihydro-5H-pyrrolizine (II). (2) The bis-trimethylsilyl derivative of lycopsamine (VII). b, Typical gas chromatogram obtained from hairpencil extracts of *D. hamatus hamatus* after trimethylsilylation using the column and conditions described in (a). (1) 1-methyl-7-oxo-6, 7-dihydro-5H-pyrrolizine (I); (2), the monotrimethylsilyl derivative of 1-formyl-7-hydroxy-6, 7-dihydro-5H-pyrrolizine (II); (3), the bis-trimethylsilyl derivative of lycopsamine (VII). (see Fig. 1).

indicine⁹ (IV), a diastereoisomer of lycopsamine. These plants are now under investigation, as possible sources of the hairpencil alkaloid.

The requirement by male butterflies of the subfamily Danainae for pyrrolizidine alkaloids is an extraordinary fact which we believe is a pointer to their evolutionary development.

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¹ Meinwald, J., Meinwald, Y. C., Wheeler, J. W., Eisner, T., and Brower, L. P., *Science, N.Y.*, **151**, 583 (1966).

- ² Meinwald, J., Meinwald, Y. C., and Mazzocchi, P. H., *Science*, N.Y., **164**, 1174 (1969).
- ³ Edgar, J. A., Culvenor, C. C. J., and Smith, L. W., *Experientia*, **27**, 761 (1971).
- ⁴ Meinwald, J., Thompson, W. R., Eisner, T., and Owen, D. F., *Tetrahedron Lett.*, **3485** (1971).
- ⁵ Edgar, J. A., Culvenor, C. C. J., and Robinson, G. S., *J. Aust. ent. Soc.*, **12**, 144 (1973).
- ⁶ Pliske, T. E., and Eisner, T., *Science*, N.Y., **164**, 1170 (1969).
- ⁷ Mattocks, A. R., *Nature*, **217**, 723 (1968).
- ⁸ Jago, M. V., Edgar, J. A., Smith, L. W., and Culvenor, C. C. J., *Molec. Pharmac.*, **6**, 402 (1970).
- ⁹ Bull, L. B., Culvenor, C. C. J., and Dick, A. T., *The Pyrrolizidine Alkaloids* (North-Holland, Amsterdam, 1968).
- ¹⁰ Culvenor, C. C. J., and Smith, L. W., *Aust. J. Chem.*, **19**, 1955 (1966).

Population estimates from recapture studies in which no recaptures have been made

THE number of animals in a population can be estimated from the proportion of individuals in a sample which have been captured previously¹⁻³. Animals which have been captured previously may be recognised by some method of marking. If the population is very large relative to the size of the samples, no marked individuals might be recaptured, because the probability of capturing any of the few marked animals is very small. Marked animals may, however, fail to be recaptured even if the population is quite small, because of random sampling error. It has not been realised before that a useful estimate of population size can be made even when no animals have been recaptured.

Suppose that a animals from a total population of size N have been marked and released. On a subsequent occasion, n animals are captured, none of which bear marks. If it is assumed that the usual conditions of recapture analysis are met, then the probability that the first animal to be captured will be unmarked is $(N - a)/N$. Similarly, the probability that the second animal to be captured is unmarked is $(N - a - 1)/(N - 1)$. Thus, the probability that all n captures are unmarked is:

$$\begin{aligned}
 P &= \frac{(N - a)}{N} \cdot \frac{(N - a - 1)}{(N - 1)} \cdots \frac{N - a - (n - 1)}{N - (n - 1)} \\
 &= \frac{(N - a)! / (N - a - n)!}{N! / (N - n)!} \\
 &= \frac{(N - a)! (N - n)!}{N! (N - a - n)!}
 \end{aligned}$$

By inserting trial values of N into this equation, one can obtain the probability P that the population is not larger than this trial value. The distribution of the probability is shown in Fig. 1, for an example in which eight marked animals have been released and several animals have subsequently been captured, none of which bear marks. As expected, the curves become asymptotic with $N = \infty$ at $P = 1$ and with $N < (n + a)$ at $P = 0$. I would suggest that in studies of small populations, an appropriate minimum estimate of population size may be made by solving the equation given above for N , by iteration, with $P = 0.5$. The meaning of this estimate is that the probability that the population

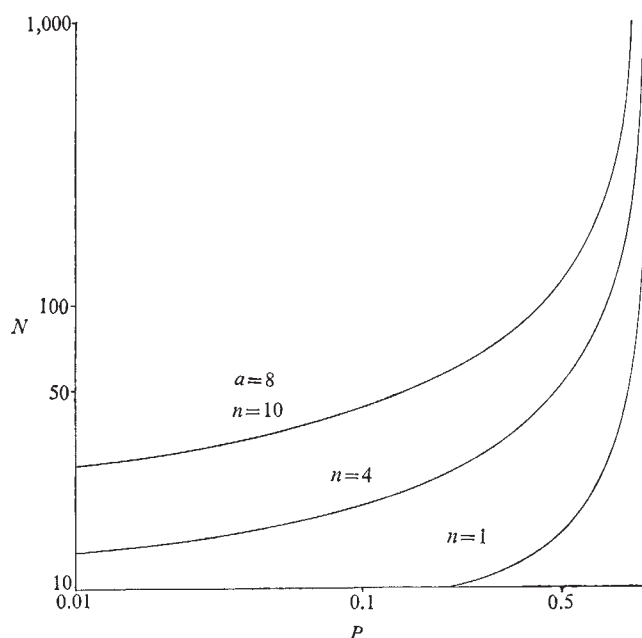


FIG. 1 Probability distribution for an example described in the text.

is larger than the estimate is equal to the probability that it is smaller. If 95% limits are required, they can be obtained by solving for N with $P = 0.025$ and $P = 0.975$ respectively.

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¹ Lincoln, F. C., *U.S. Department of Agriculture Circ.*, **118**, 1-4 (1930).

² Fisher, R. A., and Ford, E. B., *Heredity*, **1**, 143-174 (1947).

³ Jolly, G. M., *Biometrika*, **50**, 113-128 (1963).

Restoration of libido in castrated red deer stag (*Cervus elaphus*) with oestradiol-17 β

THE role of oestrogens in male reproduction remains an enigma. It is known that the stallion^{1,2} and the boar³ excrete enormous amounts of oestrogen in their urine, and that this is of testicular origin^{4,5}; more modest amounts of oestrogen are secreted by the testis of the bull⁶, rat⁷, and man⁸⁻¹⁰. Interest in male oestrogen was recently reawakened by the discovery that the hypothalami of rats, rabbits, monkeys and men are capable of aromatising testosterone to oestradiol-17 β (ref. 11). This indicated that oestradiol-17 β might be the central mediator of androgenic effects, an idea that is beginning to receive support from a number of different experiments. For example, it has been established that newborn female rats can be sterilised equally effectively by small doses of either androgen or oestrogen¹². In addition, the anti-oestrogen MER-25 will effectively inhibit this neonatal sterilisation even when it has been induced by testosterone¹³. It also seems significant that androgens which cannot be metab-

olised to oestrogen, such as dihydrotestosterone (DHT), have no apparent effect on the brain¹³⁻¹⁶, although they are potent stimulators of male secondary sexual characteristics. Whilst DHT alone cannot restore libido to a castrated male rat, the addition of a few microgrammes of oestradiol will restore libido completely^{17,18} and, recently, it has been shown that pharmacological doses of oestradiol benzoate are sufficient to restore all components of male reproductive behaviour to castrate male rats²⁰.

We decided to investigate the effects of oestradiol-17 β on the social and sexual behaviour of free-living, wild red deer stags on the island of Rhum, off the west coast of Scotland. Although red deer may seem rather unusual experimental animals, they are particularly suitable for this type of investigation for a number of reasons. Changes in social hierarchy can easily be quantitated within the bachelor group in which stags associate for most of the year^{21,22}. Sexual behaviour is virtually confined to the rut in September and October, in which the final act of copulation is preceded by several weeks of elaborate and well-defined behavioural displays. These include roaring, flehmen, wallowing in peat hags, thrashing the herbage with the antlers, repeated 'flicking' of the penile sheath, protrusion of the penis with emission of spurts of urine which soak the ventral body wall and impart the characteristic strong rutting odour, migration from the home range to a traditional rutting area, herding of groups of hinds, and intense aggressive displays frequently leading to fights with other stags^{21,22}. Testosterone appears to exert a dose-dependent inductive effect on the level of social aggression, whereas it has a permissive effect on sexual behaviour, which can only be exhibited at certain times of the year²². Castration of a stag even only a few weeks before the rut will eliminate every component of sexual behaviour, which can be fully restored with an implant of testosterone²². Finally, the antlers of the stag serve as a convenient external indicator of its endocrine state, as antler casting in the spring is a result of a testosterone deficiency, and can be produced experimentally by castration, whereas cleaning of the velvet from the growing antler in late summer is a consequence of rising testosterone levels²².

We carried out experiments on two intact and three castrated adult stags, each of which was given a subcutaneous implant of 100 mg oestradiol-17 β (Organon) at various times of the year; castrate and intact animals were implanted in early January and late August, and another castrated animal was implanted in early July.

The intact stags started to roar about 3 weeks after receiving the implants, and they kept this up for many months, whereas untreated normal stags never roar outside the rut. There was no initial change in the social rank of the implanted animals, and at no time did they show any signs of oestrous behaviour²³, or attract the sexual attention of other stags. They rutted normally at the usual time, but they failed to cast their antlers for at least 2 yr, giving some indication of the longevity of the implants. They gained a temporary advantage over normal stags when the latter were

casting their old antlers and growing new ones in velvet, and so they rose temporarily in the hierarchy.

The castrated animals responded to the implants by cleaning the velvet from their antlers 3 to 4 weeks later, and starting to roar. They then went on to show most of the components of rutting behaviour, including flehmen, wallowing, thrashing, flicking the penile sheath, and urination from the protruded penis. They also began to herd hinds in a manner characteristic of a rutting stag, and displayed and fought with other stags. They failed to develop a male rutting odour, however, and failed to migrate out of their home range, nor did they sustain their rutting activity for more than a few hours at a time.

Because of the practical difficulties in observing the mating behaviour of the castrates in the wild, one castrate was implanted with oestrogen whilst confined to a 15 hectare fenced enclosure on Rhum. This animal had spent a number of years in the enclosure whilst intact but vasectomised, when he had shown full normal libido. Following castration in August 1972, all components of male sexual behaviour immediately disappeared, and he cast his antlers and even became subordinate to a group of hinds in the same enclosure. He was implanted with 100 mg oestradiol-17 β on July 8, 1973, and cleaned his antlers during the first week of August. He then rapidly reasserted his dominance over all the hinds, but remained subordinate to two intact stags. When these stags were removed from his enclosure, the castrate immediately took possession of the hinds, showing the complete repertoire of rutting behaviour including mounting of hinds in oestrus, intromission, and ejaculation. At the height of the rut in October the castrate engaged in a vicious and prolonged fight with one of the intact stags that had been allowed temporary access to his enclosure.

To establish whether this resurgence of sexual activity was a direct effect of the oestrogen implant, or a result of androgen secretion by the adrenal glands or a testicular rest, the castrate was killed on October 22 and subjected to a detailed post-mortem examination; the results were compared with those from normal stags and an untreated castrate killed at the same time of year²⁴ (Table 1). Peripheral blood samples were collected and oestradiol-17 β and testosterone measured by radioimmunoassay. The weight of the oestradiol implant at autopsy was 89.4 mg, indicating that a total dose of 10.6 mg had been absorbed over a period of 106 d, giving an average daily release of 100 μ g. The concentration of oestradiol in the peripheral blood of this animal was 38 pg ml⁻¹, whereas it was undetectable (< 5 pg ml⁻¹) in the peripheral blood of an intact stag during the rut.

No testosterone could be detected in the peripheral blood of the castrate (<0.1 ng ml⁻¹), in striking contrast to the levels in intact rutting animals (8.9 ng ml⁻¹), so it seems safe to conclude that the rutting behaviour of the castrate was indeed produced by oestradiol-17 β (or some metabolite) acting directly on the brain. The oestrogen had also apparently caused some stimulation of the seminal vesicles and limited fructose synthesis, but it had completely failed

TABLE 1 Comparison of hormone levels and secondary sexual characteristics of intact stags, a castrate stag, and an oestrogen implanted castrate stag in October.

	Combined weight of seminal vesicles (g)	Fructose content of seminal vesicles (mg/100 g)	Peripheral plasma oestradiol-17 β (pg ml ⁻¹)	Peripheral plasma testosterone (ng ml ⁻¹)	Neck girth (cm)	Mane length (cm)	State of antlers	Presence of rutting odour
Intact stags	68* (4)	285* (4)	< 5	8.9* (5)	64* (6)	10* (6)	Hard horn	+++
Castrate stags ²⁴	8	30			50	4	Velvet	—
Oestrogen implanted castrate stag	26	82	38	< 0.1	55	6	Hard horn	—

* indicates that the measurement is a mean, figures in parentheses indicate sample size²⁴.

to stimulate the development of the rutting odour, and the neck mane and neck musculature were only slightly developed.

In conclusion, this study has demonstrated that in the red deer stag, microgramme quantities of oestradiol-17 β are as effective as milligramme quantities of testosterone in restoring full sexual behaviour to castrated animals, and in producing antler changes. But unlike testosterone, oestrogen does not seem to have a direct effect on the induction of social aggression. Neither hormone is capable of producing sexual activity out of season in intact stags.

It remains to be discovered for the generality of mammals whether testicular oestrogens or oestrogens formed from testosterone in the central nervous system are essential for eliciting components of male sexual behaviour.

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¹ Haussler, E. P., *Helv. chim. Acta.*, **17**, 531 (1934).

² Zondek, B., *Nature*, **133**, 209 (1934).

³ Velle, W., *Acta endocr. Copenh.* **28**, 255 (1958).

⁴ Beall, D., *Biochem. J.*, **34**, 1293 (1940).

⁵ Nyman, M. A., Geiger, J., and Goldzieher, J. W., *J. biol. Chem.* **234**, 16 (1959).

⁶ Velle, W., *Nature*, **180**, 856 (1957).

⁷ De Jong, F. H., Hey, A. H., Van Der Molen, H. J., *J. Endocr.*, **57**, 277 (1973).

⁸ Leach, R. B., Maddock, W. O., Tokuyama, I., Paulsen, C. A., and Nelson, W. O., *Recent Prog. Horm. Res.*, **12**, 377 (1956).

⁹ Fishman, L. M., Sarfaty, G. A., Wilson, H., and Lipsett, M. B., *Ciba Fdn. Colloq. Endocr.*, **16**, 156 (1967).

¹⁰ Baird, D. T., Galbraith, A., Fraser, I. S., and Newsam, J. E., *J. Endocr.*, **57**, 285 (1973).

¹¹ Ryan, K. J., Naftolin, F., Reddy, V., Flores, F., Petro, Z., *Am. J. Obstet. Gynec.*, **114**, 454 (1972).

¹² Brown-Grant, K., *Proc. Sir Joseph Barcroft Centenary Symp.*, 527 (Cambridge University Press, 1973).

¹³ McDonald, P., Beyer, C., Newton, F., Brien, B., Baker, R., Tan, H. S., Sampson, C., Kitching, P., Greenhill, R., and Pritchard, D., *Nature*, **227**, 964 (1970).

¹⁴ Feder, H. H., *J. Endocr.*, **51**, 214 (1971).

¹⁵ Whalen, R. E., and Lutttge, W. G., *Horm. Behav.*, **2**, 117 (1971).

¹⁶ Johnston, P. and Davidson, J. M., *Horm. Behav.*, **3**, 345 (1972).

¹⁷ Baum, M. J., and Vreeburg, J. T. M., *Science, N.Y.*, **182**, 283 (1973).

¹⁸ Larsson, K., Södersten, P., and Beyer, C., *J. Endocr.*, **57**, 563 (1973).

¹⁹ McDonald, P. G., and Doughty, C., *J. Endocr.*, **55**, 455 (1972).

²⁰ Södersten, P., *Horm. Behav.*, **4**, 247 (1973).

²¹ Lincoln, G. A., Youngson, R. W., and Short, R. V., *J. Reprod. Fert.*, Supplement 11, 71 (1970).

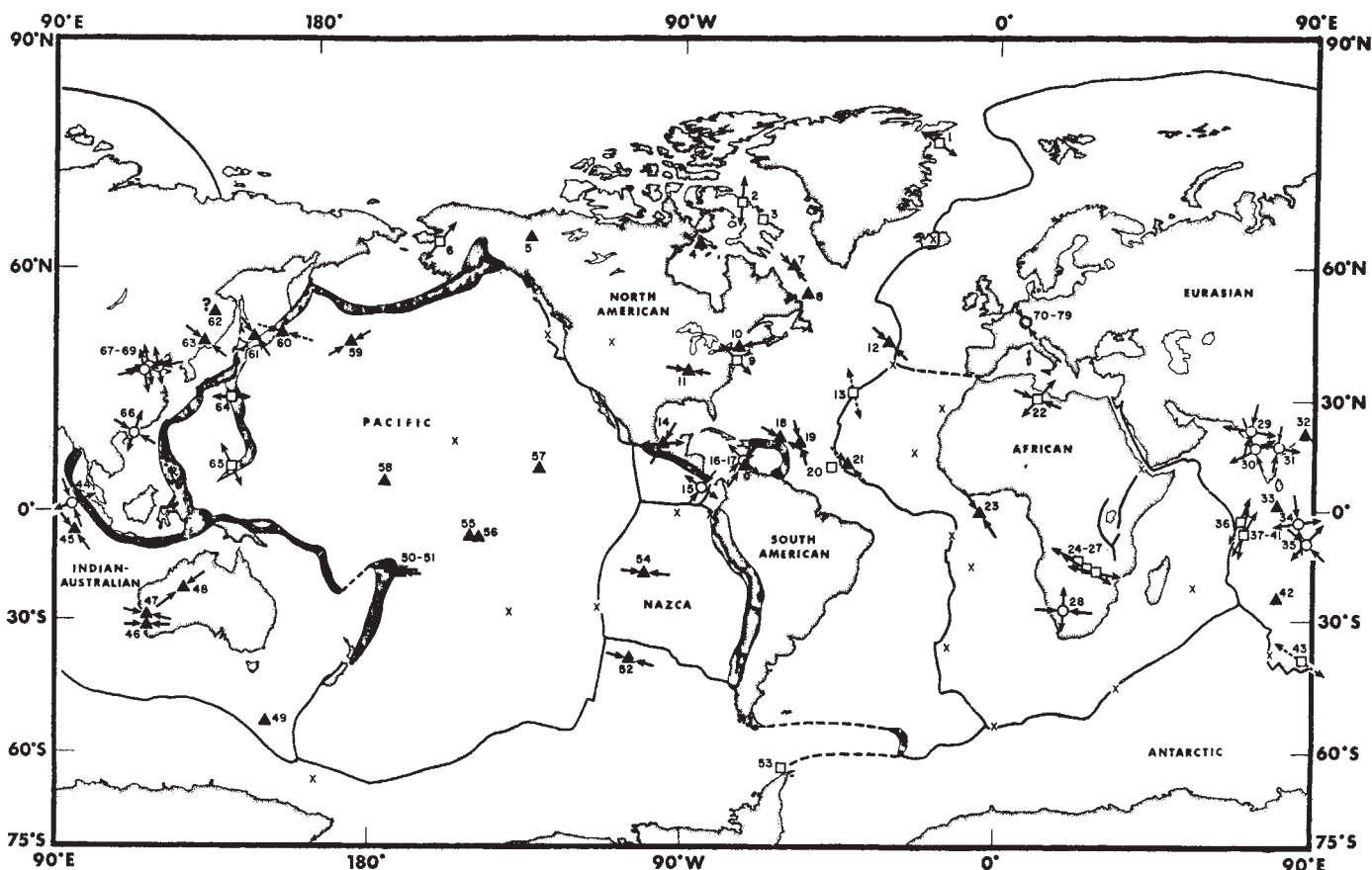
²² Lincoln, G. A., Guinness, F., and Short, R. V., *Horm. Behav.*, **3**, 375 (1972).

²³ Guinness, F., Lincoln, G. A., and Short, R. V., *J. Reprod. Fert.*, **27**, 427 (1971).

²⁴ Lincoln, G. A., *J. Zool. Lond.*, **163**, 105 (1971).

Erratum

IN the article "Intraplate Earthquakes, Lithospheric Stresses and the Driving Mechanism of Plate Tectonics" by Lynn R. Sykes and Marc L. Sbar (*Nature*, **245**, 298; 1973), Fig. 3 was reproduced on too small a scale for the salient points to be clear and so it is reproduced again here.



book reviews

Essays on Needham and China

Chinese Science: Explorations of an Ancient Tradition. Edited by Shigeru Nakayama and Nathan Sivin. Pp. xxxvi+334. (MIT East Asian Science Series, Vol. 2.) (MIT: Cambridge, Mass and London, 1973.) £5.65.

THE purpose of this compilation of essays, according to the preface prepared by the second editor (Sivin) is twofold: to provide perspectives on the work of Joseph Needham, to whom the volume is dedicated, and to provide a sample of representative 'explorations' of Chinese science. In offering to relieve the reviewer of the burden of reading each essay, the editor invites evaluation of his book in terms of the purposes he sets out.

Because the history of Chinese science and technology has been dominated in the West by the work of Joseph Needham, the efforts to put it in perspective have been few, indeed. The measure of *Science and Civilisation in China* is best taken in terms of its purpose, which Needham himself describes in his essay in the present volume:

"One of the greatest needs of the world in our time is the growth and wide dissemination of a true historical perspective, for without it whole peoples can make the gravest misjudgments about each other. Since science and its applications dominate so much our present world, since men of every race and culture take so great a pride in man's understanding of Nature and control over her, it matters vitally to know how this modern science came into being . . . distinctively modern science did in fact come into being only in Western Europe during the 'scientific revolution' of the fifteenth and sixteenth centuries, culminating in the seventeenth. But this is indeed far from being the whole story, and to tell this part of it alone is to be deeply unjust to the other civilizations."

Needham has begun a preliminary exploration, according to the present editor, putting forward tentative hypotheses, rather than providing a definitive summary of the Chinese scientific and technical traditions. The first three essays evaluate Needham's work from different perspectives. To Derek de Solla Price, it is a "definitive classic", a systematic presentation of the raw material for generations of scholars and a point of departure for all sub-

sequent work. Shigeru Nakayama analyses the elements in Needham's thought in relation to his personal life and his views as a biochemist, which have influenced his interpretation of the history of Chinese scientific and technical development: an evolutionary rather than a mechanistic viewpoint; a preference for synthesis over analysis; and dialectical materialism. These, in Nakayama's view, have led to "a number of insecurely based speculations", which contradict both conventional scholarship and historical facts. Summarising the views of Needham's critics and analysing the more controversial of Needham's views, Nakayama evaluates *Science and Civilisation in China* as a body of "heuristic suggestions and unelaborated ideas", which remain to be synthesised by future scholars. It is in this sense that Needham's work is a "determinant" of modern awareness of China. A. C. Graham comments on the development of Needham's response to the problem of why there was a Scientific Revolution in Europe but not in China, from a "fairly straightforward Marxist answer influenced by the early Wittfogel" [in *On Science and Social Change* (1944)] to "his sociological explanation but in a much more developed and refined form, for which he acknowledges a debt to Jean Chesneaux and Andre Haudricourt". Needham's thought on Chinese scientific development no more springs from a single origin than the modern science whose roots he traces to a number of cultures and societies.

The remaining six essays, by Mitukuni Yosida, Kiyosi Yabuuti, A. C. Graham and Nathan Sivin, Ho Peng Yoke, Beda Lim and Francis Morsingh, William C. Cooper and Nathan Sivin, and Saburo Miyasita, represent current scholarship based, like Needham's, on the use of primary documents, of "high intellectual and critical standards", in the editors' view. The body of scholarship which can meet such criteria is admittedly small, though its composition is ecumenical. An important contribution of this volume is in presenting representative works by Japanese scholars, which until now have rarely been accessible to the non-specialist in English translation. It is, as the editors acknowledge, regrettable that contemporary scholarship in China itself is not represented in this collection, but it is also to be regretted that the

reasons for this lack and, indeed, the present state of Chinese scholarship in this field are not discussed in the preface.

For the layman about to begin his own explorations in Chinese science, these papers provide a frame of reference for the monumental work of Joseph Needham by "de-mythologizing" the role of one man in studies of the science and technology of traditional China. With this collection of essays as a beginning, and further guided by the introductory bibliography with which this volume concludes, the layman's understanding of Chinese science should no longer have to be "determined" by Needham's work to the same extent. On the other hand, one of the motivations for the compilation of these essays was the editor's view that "everyone who is concerned about the central problems of our time has a stake in the deeper understanding of science in ancient China". Of the scholarship which deepens understanding of traditional Chinese science, it is surely that of Joseph Needham which extends understanding to those whose concern is the present.

G. C. DEAN

Fish chromosomes

Fish Chromosome Methodology. By Thomas E. Denton. Pp. vii+166. (Thomas: Springfield, Illinois, 1973.) n.p.

THE chromosomes of fishes are, characteristically, small in size and large in number. For these reasons they are difficult to handle and this book represents, in the main, a survey of cytological methods for the preparation and study of fish chromosomes.

One may well ask what justification there is for a book devoted to fish chromosomes alone. First, from a practical standpoint, there is unquestionably a big future in fish breeding for which a thorough knowledge and understanding of the chromosomes will be essential; particularly, as seems likely, where hybridisation between species may prove useful. Second, from a strictly cytological standpoint, there are many reports among fishes of Robertsonian changes associated with divergence in chromosome number between species within genera, between and within populations of the same species and even between somatic cells within individuals. It is by no means clear whether such widespread chromosome numerical variation,

in the Salmonidae for example, reflects some special structural organisation of the chromosomes facilitating breakage and rejoining in centromeric regions or, alternatively, is the consequence of special and intensive kinds of selection pressures which confer high premium upon change in linkage relations within complements.

Until the techniques for investigating fish chromosomes are improved these and other questions will remain unanswered. As the author implies the most promising development is the culture of leukocytes, along the lines which revolutionised mammalian cytology during the past two decades. So far the technique has proved only partly successful with fishes. In the meantime the book gives a comprehensive account of the many other techniques available. Also useful is the extensive list of chromosome numbers, involving 481 species. For the rest the book is a bit of a hotch-potch, dealing with subjects ranging from elementary cytology ("autosomes are composed of two strands or chromatids joined together by a centromere") to the use of the light microscope and the evolution of the fish karyotype. For those working on fish chromosomes the survey of methods, the chromosome list and accompanying references are, in themselves, good reasons for buying the book.

H. REES

Skin and feather

Avian Anatomy: Integument. By Alfred M. Lucas and Peter R. Stettenheim. Part 1 pp. iv+1-340; part 2 pp. x+341-750. (Agriculture Handbook 362: US Department of Agriculture, in cooperation with Michigan Agricultural Experiment Station, Michigan State University.) (US Government Printing Office: Washington, DC, 1972.) \$13 per 2 part set.

THE authors of this work point out that "even today there has not yet been published a complete description of any species of bird, either wild or domestic". It was to fill this gap, for the benefit of pathologists working on poultry diseases, that the basic studies began, nearly 30 years ago, of which these two volumes are an outcome. The Avian Anatomy Project, of which Professor Lucas is director, plans to issue a series of handbooks covering all organ systems. If the standard of those that are to come equals the first, this long-continued and costly undertaking will be fully justified.

There have necessarily had to be some limitations in the scope of the work. The main limitation is that, although the treatment of descriptive anatomy is full and critical, no attempt has been made at a full and critical treatment of special problems confront-

ing physiologists, pathologists and general ornithologists who will use these volumes for reference. The subject matter of the two volumes under review is also limited: treatment of skeletal muscles, ligaments, nerves, blood vessels and lymphatics is reserved for a later volume. The greater part of these two volumes is in fact devoted to pterylosis and ptilosis (the difference in the meaning of the two terms is carefully defined and discussed), feather structure (macroscopic and microscopic), feather development and growth, and other skin derivatives. The birds mainly dealt with, in addition to the domestic fowl, are turkey, Pekin duck, *Coturnix* and common pigeon; but the great horned owl is also treated in some detail and there are plenty of references to other birds.

The mass of detail that is provided is made digestible, indeed very pleasantly and easily digestible, by the clarity with which it is presented, both in the text and in the figures, and by the care that has been taken to subdivide and index it. A great deal of care has been taken to devise a consistent and clear terminology, and I hope that it will be followed by all subsequent research workers in the field. A valuable final section on methods includes a most useful introduction to techniques of anatomical illustration.

The two volumes are well produced and excellently illustrated with a total of 422 figures, some in three or four colours. By present standards the price is remarkably low, so that many of those who are most likely to need them should be able to buy them. All zoological libraries should possess a copy. As the authors point out in their introduction, "Descriptive anatomy, well done, does not become obsolete".

D. W. SNOW

Spectroscopy for rocks

Mössbauer Spectroscopy: An Introduction for Inorganic Chemists and Geochemists. By G. M. Bancroft. Pp. xii+252. (McGraw-Hill: Maidenhead, September 1973.) £6.95.

ABOUT two and a half years ago, a comprehensive review on Mössbauer spectroscopy was published. (N. N. Greenwood and T. C. Gibb, *Mössbauer Spectroscopy*, Chapman and Hall, London, 1971). Now, Professor G. M. Bancroft has published a textbook for undergraduates or postgraduates who are following courses in this subject. As the title suggests, the bias is towards applications of the technique to inorganic chemistry and geochemistry. This textbook for undergraduates and postgraduates is an effective complement to Greenwood and Gibb. At present not

many schools of chemistry provide courses in this subject but wherever Mössbauer spectroscopy is taught, the publication of a student's text will be welcomed.

The book commences with a chapter in which essential background material is presented and explained. The topics covered here include radioactivity, nuclear properties, the Doppler effect, and an account of the discovery of the Mössbauer effect. This short chapter is followed by a longer one dealing with the parameters that can be measured by Mössbauer spectroscopy. The isomer shift is introduced and explained and an account of the quadrupole interaction is given. Then magnetic hyperfine splitting is elucidated and a survey is given of the characteristic features of a useful Mössbauer isotope. This is followed by a chapter on how Mössbauer spectra may be obtained. Here one can find details of drives, sources, detectors, counting statistics, calibration, and computation of parameters using least-squares fitting programs. The use of Mössbauer spectroscopy as a fingerprint technique in inorganic chemistry is then discussed. This chapter is a set of case histories of successful applications of Mössbauer spectroscopy. Each example is carefully and critically explained.

From the earliest days of Mössbauer spectroscopy the variation in the precise energy of the absorption has been of use in understanding variations in electron density from compound to compound and the factors influencing this variation. In this book this parameter is called the centre shift and chapter 5 is devoted to a discussion of its correlation with the oxidation state of the Mössbauer element and the bonding scheme appropriate to it. Here and elsewhere in this book the author has gone to a lot of trouble to find illustrative examples involving as many different Mössbauer nuclides as possible.

Chapters 6 and 7 are the longest in the book and taken together comprise its hard core. The first of these concerns the relationship between the quadrupole splitting and the stereochemical arrangement of the groups about the absorbing atom. The second deals with the contribution made with the help of Mössbauer spectroscopy to a knowledge of the structure of rocks and minerals which contain iron. These chapters strongly reflect the research interests of the author: they are accordingly very detailed and comprehensive.

The remaining two chapters deal with the determination of site populations in silicate minerals and with the study of multi-phase assemblages such as lunar soils and rocks.

Each chapter incorporates a set of problems to which partial answers are provided. The book is well provided

with references. There are some useful appendices and an adequate index.

Research students using Mössbauer spectroscopy will find this book much more helpful than anything else available. It can be recommended without reservations to students taking courses in the subject. At today's prices, it is good value.

B. W. FITZSIMMONS

Past and present plants

Vegetation and Vegetational History of Northern Latin America. Edited by Alan Graham. (Papers presented as part of a symposium at the American Institute of Biological Sciences Meetings, Bloomington, Indiana, 1970.) Pp. xii+393. (Elsevier: Amsterdam, London and New York, 1973.) Dfl. 120; \$46.20.

THIS attractive volume does not, as its title suggests, cover the whole area between Mexico and Peru. Most of the material concerns Mexico, Panama and the Antilles, regions being actively studied by the workers whose papers are published here. That there is much more information for the whole region is shown by Graham's extremely valuable bibliography, which lists some 960 references dealing with the vegetational history of Latin America, and some review of the current situation throughout the area of the title would have been welcome.

Nevertheless, the book is a most rewarding mine of up to date information and ideas. The descriptions of modern vegetation illustrate several approaches, ranging from Porter's largely sociological survey of plant communities in Panama to the structural treatment of vegetation types in Vera Cruz by Gómez-Pompa, who furnishes a surprisingly useful dichotomous key for their identification and relates them to the prevailing ecological factors. Breedlove provides an elegant account of Chiapas, floristically the richest and most diverse of the Mexican provinces, relating the vegetation types to Beard's broader classification for tropical America, and Howard also describes the vegetation of the Antilles in structural terms. Howard further provides a statistical analysis of the distributions of taxa to illustrate the floristic groupings within the flora, particularly the different extra-Caribbean affinities of the floras of the Greater and Lesser Antilles. Similar approaches are used by Rzedowski to examine the relationships of the floras of arid regions in Mexico.

In some ways the palaeobotanical papers are more stimulating since they seriously consider the flaws in some current assumptions and techniques. Dilcher uses his incisive studies of the Middle Eocene floras of the Mississippi

delta to examine ways of overcoming the difficulties in deducing past climates and vegetation from the tolerances attributed to modern plants and points to the incorrect taxonomic affinities ascribed to many fossil floras, a topic repeatedly mentioned in the palaeobotanical papers. Barlett and Barghoorn, in describing climatic and vegetational changes in Panama during the past 12,000 years, summarise the controversy about evaluating changes in sea-level and demonstrate the greater difficulties of deducing climatic fluctuations from complex tropical vegetation as compared with the post-glacial history of mono-climax areas in North Temperate regions. Despite such problems, the power of palaeobotanical studies for tracing the history of modern floras is exemplified by Graham's demonstration that such temperate trees as *Alnus* and *Juglans*, now of disjunct occurrence through northern Latin America, gradually migrated southwards from the south-eastern United States, where they date from the Eocene, to reach northern South America by the latest Pliocene. Unfortunately, the vexed question of the means of dispersal is not discussed.

The book is well produced, excellently illustrated and, unhappily, boldly priced. It deserves to be widely read.

D. M. MOORE

Electronics made easy

Electronics in the Life Sciences. By Stephen Young. Pp. 198. (Macmillan: London and Basingstoke, August 1973.) £5.50 boards; £2.75 paper.

WHEN I visited Stephen Young in his laboratory in Edinburgh a few years ago, I was first struck by the size of his Faraday cage, and then, on entering the cage, by the variety of superbly engineered home-built equipment that he was using to record the eye movements of a small crustacean. Here was an engineer as well as a scientist, a man who took as much pleasure in designing and building equipment as he did in carrying out experiments. Now he has written a book, and a remarkably good book it is too, in which he explains, in a down-to-earth practical manner, what every good biologist should know about electronics. This approach may upset purists in the field of electronics, but it is just right for experimental biologists.

The book is thoroughly up to date with many of the circuits described incorporating integrated circuits, operational amplifiers and field effect transistors. Everything is explained from first principles with the aid of clear simple diagrams of which there are more than 250. The few criticisms that I have all concern minor errors and omissions. An earth line is omitted from Fig. 8.15; the

circuit shown in Fig. 5.52 cannot function as shown because the integrator output is the wrong sign to trigger the comparator; one is not told how to add capacitors in series or in parallel or told how to solder delicate components; a spot on an oscilloscope screen is used as a light source in such a way that it would burn a hole in the phosphor. But these are small points and detract little from the book as a whole. Indeed the book is by far the best of its type that I have read and I thoroughly recommend it to all biologists who wish to know how to design simple electronic devices or learn about the circuits on which their electronic equipment is based.

W. J. P. BARNES

Kimberlites

Lesotho Kimberlites. Edited by Peter H. Nixon. Pp. xii+350. 70 plates. (Lesotho National Development Corporation: Maseru, Lesotho, 1973.) R13; \$19.

ALTHOUGH in the past decade there have been numerous papers describing kimberlites and their xenoliths, there has not been a comprehensive volume on kimberlites in the West since the classic treatise of A. F. Williams in 1932 on the kimberlites of South Africa. In this we have lagged behind the Russians whose spate of monographs on the Siberian kimberlites have added much to knowledge of kimberlites. Now *Lesotho Kimberlites* provides a comprehensive study of a part of the South African kimberlite province on which there were previously comparatively few data.

Included within this book are the results of studies by geologists of the Overseas Development Corporation, the United Nations Development Programme, academic geologists from South Africa, Europe and the United States, and mining company geologists from South Africa. Within the book are accounts of the geology and petrology of individual diatremes and dyke swarms, and a wealth of new geochemical and mineralogical data. Although the title of the book may give the impression that this is an account of a relatively small area, the subject matter of many of the papers is of much more than purely local interest. For example, the papers on the discrete nodules and sheared peridotites from the Thaba Putsoa pipe have implications for plate tectonics and the break-up of Gondwanaland; and the papers on the Matsoku xenoliths, the petrofabrics of the peridotite suite xenoliths, and the study on the opaque oxides of the kimberlite groundmass, will all become standard references in

the kimberlite literature from now on.

Considering that twenty-eight authors have been involved in the writing of this book, the editor has maintained a creditable consistency of style. Criticisms are that there could have been more thorough cross referencing between papers, and there is repetition of data in various tables. These are minor criticisms, however, and do little to detract from the overall merits of the book. The book will appeal more to petrologists than to economic geologists since, although some kimberlites are the host rock of diamond, there are only two small sections concerning diamond. The volume is well produced and the reproduction of most plates (many in colour) is high. The book is very good value and is recommended to anyone interested in kimberlite and upper mantle geology.

J. B. DAWSON

Basis for separation

An Introduction to Separation Science. By Barry L. Karger, Lloyd R. Snyder and Csaba Horvath. Pp. xix+586. (Wiley Interscience: New York and London, November 1973.) £9.75.

SEPARATION science is a relatively new name for an important branch of analytical chemistry and though the name has probably been introduced to brighten the image of analytical chemistry there is no doubt that operations classed as separation are much more scientifically based than most of the other activities to which the word science has recently been attached. Be that as it may, the authors of this book have made a bold and largely successful attempt to treat separation processes from a fundamental point of view, at the same time recognising the great diversity of experimental techniques which can actually be used.

Chromatography is undoubtedly the most versatile, sensitive and most widespread separation technique in use today but it is arguable whether roughly half this book should have been devoted to chromatography leaving the same space to cover all other important techniques: extraction, distillation, crystallisation, membrane separation, electrophoresis, particle fractionation and mass spectrometry. The bias towards chromatography makes this book of particular value to chromatographers as it presents a unified account of this subject within a wider framework, but it may be less valuable to the chemist primarily interested in, say, zone refining or electrophoresis.

Balance aside, the really significant contribution made by the authors is their emphasis that the same basic physico-chemical principles apply to widely differing separation techniques.

These principles are outlined in the first of the three major sections of their book which covers the thermodynamic and molecular basis of distribution equilibria, the kinetics of mass transfer by diffusion and flow, and the operational aspects of the major separation methods. In the chapters of parts 2 and 3 there are repeated back references to part 1 reinforcing this emphasis on fundamentals.

Part 2 deals specifically with techniques based upon phase and distribution equilibria, and here the authors have wisely brought in outside experts to cover distillation, solvent extraction, crystallisation, ion exchange and exclusion processes, they themselves dealing with gas chromatography, liquid-liquid chromatography and liquid-solid adsorption chromatography. In part 3 they cover barrier separation processes, electrophoresis, particle separation and, very briefly, mass spectrometry.

Parts 2 and 3 present a series of reviews of uniformly high quality which contain enough theory for the basis of each technique in fundamental physical chemistry to be clear. Yet the theory does not obtrude and the book is most readable for all of its nearly 600 pages. Each chapter contains a useful bibliography listing full-length texts and the numerous original papers cited in the text.

As a chromatographer, I was particularly impressed by the authoritative and up to date treatment of the different forms of chromatography; in this respect the book is much more than an introduction and presents many new and original ideas.

This book will undoubtedly find an important place in the library of the analytical chemist and particularly in the chromatographer's, and it will provide a stimulus to the teaching of separation science. At 1.7 pence per page it is good value.

J. H. KNOX

Pyrethrum

Pyrethrum: The Natural Insecticide. Edited by John E. Casida. Pp. xvii+329. (Academic: New York and London, December 1973.) \$16; £7.70.

THIRTY years ago, a variety of insecticides of vegetable origin were in use; but virtually all of them except pyrethrum were gradually displaced as modern synthetic insecticides became available. Despite the well publicised adverse effects of these new pesticides, it is likely that they will be needed for some vital uses, for some time to come. But there is no doubt that the present climate of opinion is very favourable for pyrethrum as a non-persistent natural product; hence this book. The virtual restriction to natural pyrethrum

is perhaps unfortunate, in view of the highly promising synthetic pyrethroids recently developed in Britain.

The 17 chapters of the book examine the present knowledge of pyrethrum and its active constituents from various points of view. The first two papers, by industrial experts, trace the commercial history of pyrethrum and the present sources of production. There follow three excellent chapters on chemistry. The composition of pyrethrum extract from the flower heads and methods of analysis and assay are summarised by S.W. Head of the Pyrethrum Marketing Board of Kenya. M. Elliott and N. F. Janes then describe the stereochemistry of the active components. The existence of both geometrical and optical isomers as well as variations in structure make this a highly complex subject, relying in recent years on mass spectrometry and nuclear magnetic resonance, for confirmation of hypothetical configurations. J. Casida then deals with the biochemistry; both the synthesis of pyrethrins in the plant and their degradation pathways in animal tissues.

The next six papers deal with toxicology and pharmacology. These comprise summaries of information on, first, toxicity to mammals, then an excellent paper on the effects of synergists in regard to toxicity by I. Yamamoto, and a rather disappointing account of what is known of the actual mode of action. The three other papers in this section describe original investigations of the effects of pyrethrins on wildlife; some tests of its possible teratogenic, carcinogenic, mutagenic and allergenic action; and interactions with other drugs. In the following section, four chapters describe types of usage: for medical and veterinary pests, for agricultural, and for forestry insects. A chapter on residue and tolerance considerations (including two synergists as well as pyrethrum) is, for some reason, set in the "Summary" section. More appropriately, E. M. Mrak concludes by summarising the advantages and disadvantages of pyrethrum, leaning rather heavily towards the former.

Despite inevitable inequality of treatment, the book provides a good distillation of many complex matters as well as a useful source of references.

J. R. BUSVINE

Corrigendum

In the book review "IQ and inequality" by Jinks and Eaves (248, 287; 1974), the sentence starting in paragraph 8, line 7 should read "Making *E*, applicable purely to offspring results in a significantly poorer fit to Jencks's data" instead of "...slightly poorer...".